Table 37. Mutations known to attenuate dengue type 4 virus and the corresponding wildtype amino acid residue in other dengue virus.

	Mutation	Amino acid position[a]	Mutant residue	Amino acid in indicated wt dengue virus[b]			
				DEN4	DEN1	DEN2	DEN3
5-FU mutations	2650	850	S	N[d]	N	N	N
	3442	1114	G	E	E	E	E
	3540	1147	K	E	E	E	E
	3575	1158	I	M	L	A	M
	3771	1224	G	R	R	K	R
	4062	1321	A	T	L	A	T
	4306	1402	S	N	E	D	D
	4891	1597	T	I	V	I	I
	4896	1599	S	A	A	A	A
	4907	1602	F	L	L	L	L
	4995	1632	P	S	S	S	N
	5097	1666	N	D	D	D	D
	5695	1865	G	D	D	D	D
	6259	2053	A	V	V	V	V
	7129[c]	2343	L	P	P	P	P
	7849	2583	I	N	K	N	K
	8092	2664	G	E	Q	Q	Q
	10186	3362	T	I	I	I	I
	10634	3' UTR	-	-	-	-	-
Charge-cluster-to-alanine mutations	22, 23	2509, 2510	AA	RK	KS	KS	RK
	23, 24	2510, 2511	AA	KE	SE	SE	KE
	157, 158	2644, 2645	AA	EE	EE	EA	EE
	200, 201	2687, 2688	AA	KH	KH	KY	KH
	356, 357	2843, 2844	AA	KE	KE	KE	KE
	387, 388	2874, 2875	AA	KK	RN	KK	RN
	436, 437	2923, 2924	AA	DK	HR	DK	DK
	524, 525	3011, 3012	AA	KK	KI	KK	KI
	525, 526	3012, 3013	AA	KD	IP	KE	IP
	642, 643	3129, 3130	AA	ER	ER	IA	KK
	654, 655	3141, 3142	AA	DR	ER	ER	ER
	808, 809	3295, 3296	AA	ED	ED	ED	ED
	827, 828	3314, 3315	AA	DK	DK	DK	DK
	877, 878	3364, 3365	AA	KE	NE	NE	NE
	878, 879	3365, 3366	AA	EE	EN	EE	EE

[a] Amino acid position is given for the polyprotein of DEN4

[b] DEN4 = rDEN4 (GenBank AF326825); DEN1 = Western pacific (GenBank U88535); DEN2 = New Guinea C (GenBank AF038403); DEN3 = H87 (GenBank M93130)

[c] This mutation results in decreased replication of DEN4 in mosquitoes.

[d] Underlined nucleotides are shared between DEN4 and one or more additional DEN types.

Table 20. Temperature-sensitive and mouse brain attenuation phenotypes of viruses bearing charge-cluster-to-alanine mutations in the NS5 gene of DEN4.

Mutation[a]	Changed AA Pair	# nt changed	Mean virus titer (log_{10} PFU/ml at indicated temperature (°C)[b]										Replication in suckling mice[d]		
			Vero Cells					HuH-7 Cells						Mean titer ± SE (log_{10}PFU/g brain)	Mean log reduction from wt[e]
			35	37	38	39	Δ[c]	35	37	38	39	Δ	n		
wt (rDEN4)	*n/a*	*0*	*8.1*	*8.1*	*7.9*	*7.6*	*0.5*	*8.3*	*8.0*	*7.5*	*7.5*	*0.8*	48	6.0 ± 0.16	-
deletion (rDEN4Δ30)	*n/a*	*30*	*6.3*	*6.1*	*6.1*	*5.7*	*0.6*	*6.9*	*6.3*	*5.9*	*4.7*	*2.2*	42	5.4 ± 0.22	0.6
21-22	D R	4	7.2	6.8	6.7	6.1	1.1	7.6	7.1	7.0	4.7	2.9	6	5.0 ± 0.50	0.6
22-23	R K	4	7.0	7.8	6.9	3.7	3.3	7.6	7.6	6.5	≤1.7	>5.9	6	2.6 ± 0.19	2.9
23-24	K E	3	6.7	6.6	6.0	6.5	0.2	7.1	7.3	5.6	≤1.7	>5.4	18	4.7 ± 0.09	1.5
26-27	E E	3	7.8	7.6	6.8	4.0	3.8	8.4	8.2	7.3	4.9	3.5	6	5.7 ± 0.30	+0.1
46-47	K D	3	7.4	7.4	7.3	7.0	0.4	7.8	7.8	7.3	6.8	1.0	6	5.4 ± 0.42	0.5
157-158	E E	3	6.5	7.2	5.1	5.1	1.4	7.6	7.4	5.9	≤1.7	>5.9	6	2.8 ± 0.31	2.7
200-201	K H	4	5.3	4.6	5.3	4.1	1.2	5.6	4.9	3.7	≤1.7	>3.9	12	5.5 ± 0.45	0.8
246-247	R H	5	6.9	5.8	5.7	5.4	1.5	6.4	6.1	6.1	5.5	0.9	6	6.1 ± 0.17	+0.5
253-254	E K	4	7.1	6.9	6.8	7.0	0.1	7.9	7.5	7.6	6.8	1.1	6	6.2 ± 0.13	+0.6
356-357	K E	3	7.7	7.6	7.0	7.0	0.7	8.0	7.3	6.4	≤1.7	>6.3	6	3.5 ± 0.58	2.0
387-388	K K	5	7.7	6.1	7.0	≤1.7	>6.0	7.0	6.3	7.0	≤1.7	>5.3	6	3.1 ± 0.33	2.4
388-389	K K	5	5.1	4.5	≤1.7	≤1.7	>3.4	6.1	5.0	≤1.7	≤1.7	>4.4	6	5.0 ± 0.23	1.4
396-397	R E	4	7.0	7.3	6.5	5.5	1.5	7.5	7.6	7.5	≤1.7	> 5.8	18	5.4 ± 0.35	1.1
397-398	E E	2	7.0	7.1	7.0	3.0	4.0	8.0	7.6	7.0	≤1.7	>6.3	6	6.0 ± 0.22	0.8
436-437	D K	4	4.5	3.3	3.0	2.0	2.5	5.7	4.5	≤1.7	≤1.7	>4.0	12	2.3 ± 0.14	3.9
500-501	R E	3	6.6	6.3	5.7	2.3	4.3	7.1	6.5	≤1.7	≤1.7	>5.4	6	6.9 ± 0.49	+0.7
520-521	E E	3	5.6	4.7	4.3	≤1.7	>3.9	6.7	5.7	≤1.7	≤1.7	>5.0	6	5.2 ± 0.48	0.2
523-524	D K	4	6.6	6.3	6.3	5.8	0.8	7.1	6.6	≤1.7	≤1.7	>5.4	6	4.2 ± 0.47	1.3
524-525	K K	5	7.1	6.9	6.9	6.6	0.5	7.8	7.4	7.0	5.3	2.5	6	3.4 ± 0.54	2.1
525-526	K D	4	7.8	7.1	7.6	6.8	1.0	7.9	7.7	8.0	6.9	1.0	6	3.7 ± 0.64	1.8
596-597	K D	3	4.6	4.0	2.6	≤1.7	>2.9	5.7	4.9	4.0	≤1.7	>4.0	6	5.9 ± 0.14	0.5
641-642	K E	4	7.3	6.9	6.9	5.2	2.1	7.8	7.5	7.2	6.9	0.9	6	4.7 ± 0.45	1.2
642-643	E R	3	6.8	6.1	4.0	3.3	3.5	7.5	7.1	6.6	3.0	4.5	12	2.6 ± 0.15	3.6
645-646	E K	4	6.3	5.3	5.9	3.1	3.2	6.4	5.8	5.5	4.5	1.9	6	5.4 ± 0.51	0.2
649-650	K E	3	6.9	6.8	6.9	6.3	0.6	7.1	7.3	7.5	7.0	0.1	12	6.4 ± 0.20	+0.2
654-655	D R	4	6.3	5.7	≤1.7	≤1.7	>4.6	7.0	7.1	4.6	≤1.7	>5.3	12	1.8 ± 0.10	4.0

750-751	R E	3	7.1	7.1	6.9	5.7	1.4	7.8	6.9	6.5	5.6	2.2	6	6.0 ± 0.18	0.7
808-809	E D	3	4.6	4.1	<u>≤1.7</u>	<u>≤1.7</u>	>2.9	5.2	<u>≤1.7</u>	<u>≤1.7</u>	<u>≤1.7</u>	>3.5	6	1.8 ± 0.05	**3.1**
820-821	E D	2	6.3	6.3	5.6	<u>≤1.7</u>	>4.6	6.9	6.0	5.7	<u>≤1.7</u>	>5.2	6	5n5 ± 0.33	1.2
827-828	D K	4	6.9	6.3	6.3	5.9	1.0	7.5	6.9	5.0	<u>≤1.7</u>	>5.8	6	3.6 ± 0.76	**2.3**
877-878	K E	3	7.6	7.3	7.0	7.0	0.6	7.9	7.9	7.3	5.8	2.1	12	4.4 ± 0.65	**1.8**
878-879	E E	3	7.6	7.3	7.3	7.1	0.5	8.1	8.1	7.9	6.6	1.5	12	2.4 ± 0.10	**3.8**

[a] Positions of the amino acid pair mutated to an alanine pair; numbering starts at the amino terminus of the NS5 protein.

[b] Underlined values indicate a 2.5 or 3.5 log10 PFU/ml reduction in titer in Vero or HuH-7 cells, respectively, at the indicated temperatures when compared to permissive temperature (35°C).

[c] Reduction in titer (log10 PFU/ml) at 39°C compared to permissive temperature (35°C).

[d] Groups of six mice were inoculated i.c. with 4.0 log10 PFU virus in a 30 µl inoculum. The brain was removed 5 days later, homogenized, and titered in Vero cells.

[e] Determined by comparing mean viral titers in mice inoculated with sample virus and concurrent wt controls (n = 6). The attenuation phenotype is defined as a reduction of ≥1.5 log10 PFU/g compared to wt virus; reductions of ≥1.5 are listed in boldface.

as being useful to attenuate DEN1, DEN2, and DEN3 antigenic chimeric recombinants possessing a DEN4 vector background. Second, the phenotype is usually specified by three or more nucleotide changes, rendering the likelihood of reversion of the mutant sequence to that of the wild type sequence less than for a single point mutation, such as mutations identified in the panel of 5-FU mutant viruses. Finally, charge-cluster-to-alanine attenuating mutations are envisioned as being easily combinable among themselves or with other attenuating mutations to modify the attenuation phenotype of DEN4 vaccine candidates or of DEN1, DEN2, and DEN3 antigenic chimeric recombinant viruses possessing a DEN4 vector background.

[0137] **Charge-Cluster-to-Alanine-Mutagenesis.** The cDNA p4, from which recombinant wild type and mutant viruses were generated, has been described in Examples 1, 2, and 3 and in Figure 4. Charge-cluster-to-alanine mutagenesis (Muylaert, I.R. *et al.* 1997 *J Virol* 71:291-8), in which pairs of charged amino acids are replaced with alanine residues, was used to individually mutagenize the coding sequence for 80 pairs of contiguous charged amino acids in the DEN4 NS5 gene. Subclones suitable for mutagenesis were derived from the full length DEN4 plasmid (p4) by digestion with *XmaI*/*PstI* (pNS5A), *PstI*/*Sac*II (pNS5B) or *Sac*II/*Mlu*I (pNS5C) at the nucleotide positions indicated in Figure 4. These fragments were then subcloned and Kunkel mutagenesis was conducted as described in Examples 1 and 3. To create each mutation, oligonucleotides were designed to change the sequence of individual pairs of codons to GCAGCX (SEQ ID NO: 69), thereby replacing them with two alanine codons (GCX) and also creating a *Bbv*I restriction site (GCAGC) (SEQ ID NO: 70). The *Bbv*I site was added to facilitate screening of cDNAs and recombinant viruses for the presence of the mutant sequence. Restriction enzyme fragments bearing the alanine mutations were cloned back into the full-length p4 plasmid as described in Examples 1 and 3.

[0138] Initial evaluation of the phenotype of the 32 charge-cluster-to-alanine mutant viruses revealed a range in restriction of replication in suckling mouse brain and SCID-HuH-7 mice. To determine whether attenuation could be enhanced by combining mutations, double mutant viruses carrying two pairs of charge-cluster-to-alanine mutations were created by swapping appropriate fragments carrying one pair of mutations into a

infect Vero cell monolayers in a 96-well plate and incubated for 5 to 6 days at 35°C. Following incubation, cell culture media were removed and temporarily stored at 4°C, and the virus-positive cell monolayers were identified by immunoperoxidase staining. Terminal dilution was achieved when ≤ 25% of cell monolayers were positive for virus. Cell culture medium from a positive monolayer at the terminal dilution was subjected to an additional round of terminal dilution. Following the second terminal dilution, virus was amplified in Vero cells (75 cm^2 flask), collected and frozen as previously described.

[0142] Assays for temperature-sensitivity and mouse attenuation. Assay of the level of temperature sensitivity of the charge-cluster-to-alanine mutant viruses in Vero and HuH-7 cells and their level of replication in the brain of suckling mice were conducted as described in Example 1 and assay of the level of replication in SCID-HuH-7 mice was conducted as described in Example 3.

[0143] Charge-cluster-to-alanine mutant viruses are viable and show temperature-sensitive and mouse attenuation phenotypes. Of 80 full-length DEN4 cDNA constructs containing a single pair of charge-to-alanine mutations, virus was recovered from 32 in either Vero or C6/36 cells (Figure 5). The level of temperature sensitivity of wt rDEN4, rDEN4Δ30, and the 32 mutant viruses is summarized in Table 20. One mutant virus (645-646) was *ts* in Vero but not HuH-7 cells and 7 mutant viruses were *ts* in HuH-7 but not Vero cells. Such mutants whose temperature sensitivity is host-cell dependent are referred to as temperature-sensitive, host-range (*tshr*) mutants. Thirteen mutant viruses were *ts* in both cell types, and 11 mutant viruses were not *ts* on either cell type. Thus a total of 21 mutant viruses were *ts* with 8 mutant viruses exhibiting an *tshr* specificity. None of the mutant viruses showed a small plaque phenotype at permissive temperature. Mutant viruses showed a wide range (0 to 10,000-fold) of restricted replication in suckling mouse brain (Table 20). Fourteen mutant viruses were attenuated in suckling mouse brain, arbitrarily defined as a ≥ 1.5 $\log_{10}$–unit reduction in virus titer. There was no correlation between attenuation in mouse brain and temperature sensitivity in either Vero cells (Kendall Rank correlation: $P = 0.77$) or HuH-7 cells (Kendall Rank correlation: $P = 0.06$).

[0144] Thirteen mutant viruses that either showed an *att* phenotype in suckling mouse brain or whose unmutated charged amino acid pair was highly conserved among the

four DEN serotypes (see Example 7) were assayed for *att* in SCID-HuH-7 mice (Table 21). Three of these mutant viruses showed >100-fold decrease in replication relative to wild type DEN4. Overall, mean log reduction from wild type in suckling mice did not show significant correlation with mean log reduction in SCID-HuH-7 mice (Spearman rank correlation, N = 13, P = 0.06). However, mutant virus 200-201 was unusual in that it showed a high level of restriction in SCID-HuH-7 mice but little restriction in suckling mouse brain. When virus 200-201 was removed from the analysis, restriction of replication in suckling and SCID-HuH-7 mice showed a significant correlation (Spearman rank correlation, N = 12, P = 0.02).

[0145] Combining charge-cluster-to-alanine mutations present in two viruses into one virus can enhance its *ts* and *att* phenotypes. Six paired mutations were combined into fourteen double-pair mutant viruses, of which six could be recovered in Vero or C6/36 cells (Table 22). All of the individual paired mutations used in double-pair mutant viruses were *ts* on HuH-7 cells, none was *ts* in Vero cells, and for all combinations at least one mutation pair conferred an *att* phenotype in suckling mouse brain. Evaluation of four of the double-pair mutant viruses (Table 23) revealed that combining charge-cluster-to-alanine mutation pairs invariably resulted in the acquisition of a *ts* phenotype in Vero cells (4 out of 4 viruses) and often resulted in a lowered shutoff temperature in HuH-7 cells (3 out of 4 viruses). In half of the viruses assayed, combination of charge-cluster-to-alanine mutation pairs resulted in enhanced restriction of replication (10-fold greater than either component mutation) in suckling mouse brain (Table 23) and in SCID-HuH-7 mice (Table 24).

[0146] Summary. The major usefulness of the charge-cluster-to-alanine mutations stems from their design: they are located in the DEN4 non-structural gene region and therefore are envisioned as being useful to attenuate DEN4 itself as well as antigenic chimeric viruses possessing the DEN4 NS gene region. Furthermore, they are predicted to be phenotypically more stable than the single-nucleotide substitution mutant viruses such as the 5-FU mutant viruses. Finally, combinations of mutations are envisioned as being created in order to fine-tune attenuation and to further stabilize attenuation phenotypes.

```
* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  FEB 27  New STN AnaVist pricing effective March 1, 2006
 NEWS  4  APR 04  STN AnaVist $500 visualization usage credit offered
 NEWS  5  MAY 10  CA/CAplus enhanced with 1900-1906 U.S. patent records
 NEWS  6  MAY 11  KOREAPAT updates resume
 NEWS  7  MAY 19  Derwent World Patents Index to be reloaded and enhanced
 NEWS  8  MAY 30  IPC 8 Rolled-up Core codes added to CA/CAplus and
                  USPATFULL/USPAT2
 NEWS  9  MAY 30  The F-Term thesaurus is now available in CA/CAplus
 NEWS 10  JUN 02  The first reclassification of IPC codes now complete in
                  INPADOC
 NEWS 11  JUN 26  TULSA/TULSA2 reloaded and enhanced with new search and
                  and display fields
 NEWS 12  JUN 28  Price changes in full-text patent databases EPFULL and PCTFULL
 NEWS 13  JUl 07  Coverage of Research Disclosure reinstated in DWPI

 NEWS EXPRESS  JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS LOGIN    Welcome Banner and News Items
 NEWS IPC8     For general information regarding STN implementation of IPC 8
 NEWS X25      X.25 communication option no longer available

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

  ********************************************************************
  Due to STN maintenance on Sunday, July 9th, 2006,  some databases
  may not be available until 04:00 (4:00 AM) Eastern Daylight Time.
  ********************************************************************

* * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 01:43:33 ON 10 JUL 2006

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'USPATFULL' ENTERED AT 01:43:53 ON 10 JUL 2006
CA INDEXING COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 6 Jul 2006 (20060706/PD)
FILE LAST UPDATED: 6 Jul 2006 (20060706/ED)
HIGHEST GRANTED PATENT NUMBER: US7073200
HIGHEST APPLICATION PUBLICATION NUMBER: US2006150291
CA INDEXING IS CURRENT THROUGH 6 Jul 2006 (20060706/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 6 Jul 2006 (20060706/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2006
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2006


=> e whitehead stephen s/in
E1            3     WHITEHEAD STEPHEN CRAIG/IN
E2           77     WHITEHEAD STEPHEN P/IN
E3           14 --> WHITEHEAD STEPHEN S/IN
E4            2     WHITEHEAD STEVEN/IN
E5            4     WHITEHEAD STEVEN P/IN
E6            2     WHITEHEAD SUSAN/IN
E7            1     WHITEHEAD SUSAN A/IN
E8            2     WHITEHEAD T WILSON/IN
E9            1     WHITEHEAD THOMAS/IN
E10          10     WHITEHEAD THOMAS P/IN
E11           2     WHITEHEAD THOMAS W/IN
E12           1     WHITEHEAD THOMAS WILSON/IN

=> s e3
L1           14 "WHITEHEAD STEPHEN S"/IN
```

L1 ANSWER 1 OF 14 USPATFULL on STN
2006:66941 Production of attenuated, human-bovine chimeric respiratory syncytial virus vaccines.
Buchholz, Ursula, Insel Riems, GERMANY, FEDERAL REPUBLIC OF
Collins, Peter L., Kensington, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Whitehead, Stephen S., Gaithersburg, MD, UNITED STATES
Krempl, Christine D., Rockville, MD, UNITED STATES
National Institutes of Health, Office of Technology Transfer, Rockville, MD, UNITED STATES (non-U.S. corporation)
US 2006057158 A1 20060316
APPLICATION: US 2005-97946 A1 20050331 (11)
PRIORITY: US 1999-143132P 19990709 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 2 OF 14 USPATFULL on STN
2006:27966 Respiratory syncytial virus vaccines expressing protective antigens from promotor-proximal genes.
Krempl, Christine D., Merzhausen, GERMANY, FEDERAL REPUBLIC OF
Collins, Peter L., Kensington, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Buchholz, Ursula, Bethesda, MD, UNITED STATES
Whitehead, Stephen S., Gaithersburg, MD, UNITED STATES
The Government of the United States As Represented by the Department of Health and Human Services (U.S. government)The United States Public Health Service As Represented by the Office of Technology (U.S. corporation)
US 2006024797 A1 20060202
APPLICATION: US 2005-33055 A1 20050110 (11)
PRIORITY: US 2000-213708P 20000623 (60)
US 1999-143132P 19990709 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 3 OF 14 USPATFULL on STN
2006:21095 Respiratory syncytial virus vaccines expressing protective antigens from promotor-proximal genes.
Krempl, Christine D., Rockville, MD, UNITED STATES
Collins, Peter L., Rockville, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Buchholz, Ursula, Insel Riems, GERMANY, FEDERAL REPUBLIC OF
Whitehead, Stephen S., Gaithersburg, MD, UNITED STATES
The Govt. of the U.S.A, Department of Health and Human Services National Institutes of Health, Rockville, MD, UNITED STATES, 20852-3804 (U.S. corporation)
US 2006018927 A1 20060126
APPLICATION: US 2005-54343 A1 20050208 (11)
PRIORITY: US 2000-213708P 20000623 (60)
US 1999-143132P 19990709 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 4 OF 14 USPATFULL on STN
2005:254273 Production of recombinant respiratory syncytial viruses expressing immune modulatory molecules.
Collins, Peter L., Rockville, MD, UNITED STATES
Bukreyev, Alexander, Rockville, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Whitehead, Stephen S., Gaithersburg, MD, UNITED STATES
US 2005220767 A1 20051006
APPLICATION: US 2004-917984 A1 20040811 (10)
PRIORITY: US 1999-143425P 19990713 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 5 OF 14 USPATFULL on STN
2005:182955 Production of attenuated, human-bovine chimeric respiratory syncytial viruses for use in immunogenic compositions.
Buchholz, Ursula, Bethesda, MD, UNITED STATES
Collins, Peter L., Kensington, MD, UNITED STATES
Murphy, Brian P., Bethesda, MD, UNITED STATES
Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
Krempl, Christine D., Merzhausen, GERMANY, FEDERAL REPUBLIC OF
National Institutes of Health, Rockville, MD, UNITED STATES (U.S. corporation)
US 2005158338 A1 20050721
APPLICATION: US 2003-704116 A1 20031107 (10)
PRIORITY: US 1999-143132P 19990709 (60)
US 2000-213708P 20000623 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 6 OF 14 USPATFULL on STN
2005:170875 Production of recombinant respiratory syncytial viruses expressing immune modulatory molecules.
Collins, Peter L., Kensington, MD, UNITED STATES
Bukreyev, Alexander, Olney, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Whitehead, Stephen S., Montgometry Village, MD, UNITED STATES
The Govt. of the USA, as represented by the Department of Health and Human Services (U.S. corporation)National Institutes of Health Office of Technology Transfer, Rockville, MD, UNITED STATES (U.S. corporation)
US 2005147622 A1 20050707
APPLICATION: US 2004-754895 A1 20040108 (10)
PRIORITY: US 1999-143425P 19990713 (60)
US 1997-47634P 19970523 (60)
US 1997-46141P 19970509 (60)
US 1996-21773P 19960715 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 7 OF 14 USPATFULL on STN
2005:117619 Construction of West Nile virus and dengue virus chimeras for use in a live virus vaccine to prevent disease caused by West Nile virus.
Pletnev, Alexander G., Rockville, MD, UNITED STATES
Putnak, Joseph R., Silver Spring, MD, UNITED STATES
Chanock, Robert M., Bethesda, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
Blaney, Joseph E. JR., Frederick, MD, UNITED STATES
US 2005100886 A1 20050512
APPLICATION: US 2004-871775 A1 20040618 (10)
PRIORITY: US 2002-347281P 20020110 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 8 OF 14 USPATFULL on STN
2005:117292 Production of attenuated chimeric respiratory syncytial virus vaccines from cloned nucleotide sequences.
Collins, Peter L., Rockville, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Whitehead, Stephen S., Gaithersburg, MD, UNITED STATES
The Government of the USA, as represented by the Department of Health & Human Services. (U.S. corporation)
US 2005100557 A1 20050512
APPLICATION: US 2003-722000 A1 20031125 (10)
PRIORITY: US 1997-47634P 19970523 (60)
US 1997-46141P 19970509 (60)
US 1996-21773P 19960715 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 9 OF 14 USPATFULL on STN
2005:11907 Development of mutations useful for attenuating dengue viruses and chimeric dengue viruses.
Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Hanley, Kathryn A., Bethesda, MD, UNITED STATES
Blaney, Joseph E., Frederick, MD, UNITED STATES
US 2005010043 A1 20050113
APPLICATION: US 2003-719547 A1 20031121 (10)
PRIORITY: US 2001-293049P 20010522 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 10 OF 14 USPATFULL on STN
2004:53240 Production of recombinant respiratory syncytial viruses expressing immune modulatory molecules.
Collins, Peter L., 12304 Village Square, #401, Rockville, MD, United States 20852
Bukreyev, Alexander, 13103 Elsdale Ct., #102, Rockville, MD, United States 20851
Murphy, Brian P., 5410 Tuscawaras Rd., Bethesda, MD, United States 20816
Whitehead, Stephen S., 7 Prairie Rose La., Gaithersburg, MD, United States 20878
US 6699476 B1 20040302
APPLICATION: US 2000-614285 20000712 (9)
PRIORITY: US 1999-143425P 19990713 (60)
US 1997-47634P 19970523 (60)
US 1997-46141P 19970509 (60)
US 1996-21773P 19960715 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

2004:33872 Production of attenuated chimeric respiratory syncytial virus vaccines from cloned nucleotide sequences.
Collins, Peter L., Rockville, MD, United States
Murphy, Brian R., Bethesda, MD, United States
Whitehead, Stephen S., Gaithersburg, MD, United States
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6689367 B1 20040210
APPLICATION: US 1999-291894 19990413 (9)
PRIORITY: US 1997-47634P 19970523 (60)
US 1997-46141P 19970509 (60)
US 1996-21773P 19960715 (60)
US 1995-7083P 19950927 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 12 OF 14 USPATFULL on STN
2004:7309 Respiratory syncytial virus vaccines expressing protective antigens from promotor- proximal genes.
Krempl, Christine D, Merzhavsen, GERMANY, FEDERAL REPUBLIC OF
Collins, Peter L, Kensington, MD, UNITED STATES
Murphy, Brian R, Bethesda, MD, UNITED STATES
Buchholz, Ursula, Bethesda, MD, UNITED STATES
Whitehead, Stephen S, Gaithersburg, MD, UNITED STATES
US 2004005542 A1 20040108
APPLICATION: US 2003-312191 A1 20030701 (10)
WO 2001-US20107 20010622
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 13 OF 14 USPATFULL on STN
2002:265561 Respiratory syncytial virus vaccines expressing protective antigens from promoter-proximal genes.
Krempl, Christine D., Rockville, MD, UNITED STATES
Collins, Peter L., Rockville, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Buchholz, Ursula, Insel Riems, GERMANY, FEDERAL REPUBLIC OF
Whitehead, Stephen S., Gaithersburg, MD, UNITED STATES
US 2002146433 A1 20021010
APPLICATION: US 2001-887469 A1 20010622 (9)
PRIORITY: US 2000-213708P 20000623 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 14 OF 14 USPATFULL on STN
1999:155208 Production of attenuated respiratory syncytial virus vaccines from cloned nucleotide sequences.
Murphy, Brian R., Bethesda, MD, United States
Collins, Peter L., Rockville, MD, United States
Whitehead, Stephen S., Gaithersburg, MD, United States
Bukreyev, Alexander A., Rockville, MD, United States
Juhasz, Katalin, Rockville, MD, United States
Teng, Michael N., Rockville, MD, United States
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 5993824 19991130
APPLICATION: US 1997-892403 19970715 (8)
PRIORITY: US 1997-47634P 19970523 (60)
US 1997-46141P 19970509 (60)
US 1996-21773P 19960715 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l1,cbib,clm,7,9

L1 ANSWER 7 OF 14 USPATFULL on STN
2005:117619 Construction of West Nile virus and dengue virus chimeras for use in a live virus vaccine to prevent disease caused by West Nile virus.
Pletnev, Alexander G., Rockville, MD, UNITED STATES
Putnak, Joseph R., Silver Spring, MD, UNITED STATES
Chanock, Robert M., Bethesda, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
Blaney, Joseph E. JR., Frederick, MD, UNITED STATES
US 2005100886 A1 20050512
APPLICATION: US 2004-871775 A1 20040618 (10)
PRIORITY: US 2002-347281P 20020110 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A nucleic acid chimera comprising a first nucleotide sequence

second nucleotide sequence encoding nonstructural proteins from a dengue virus with the proviso that the dengue virus is not DEN1 PDK13, DEN2 PDK53, DEN3 PGMK30/FRhL3, or DEN4 PDK48.

2. The nucleic acid chimera of claim 1, wherein the dengue virus is dengue type 1 virus.

3. The nucleic acid chimera of claim 1, wherein the dengue virus is dengue type 2 virus.

4. The nucleic acid chimera of claim 1, wherein the dengue virus is dengue type 3 virus.

5. The nucleic acid chimera of claim 1, wherein the dengue virus is dengue type 4 virus.

6. The nucleic acid chimera of claim 1, wherein the dengue virus is an attenuated virus or a virus adapted for increased growth in Vero cells.

7. The nucleic acid chimera of claim 6, wherein the dengue virus is dengue type 4 virus and the virus is attenuated by a deletion of about 30 nucleotides from the 3' untranslated region of the dengue type 4 genome corresponding to the TL2 stem-loop structure between about nucleotides 10478-10507.

8. The nucleic acid chimera of claim 6, wherein the dengue virus is dengue type 1 virus and the virus is attenuated by a deletion of about 30 nucleotides from the 3' untranslated region of the dengue type 1 genome corresponding to the TL2 stem-loop structure between about nucleotides 10562-10591.

9. The nucleic acid chimera of claim 6, wherein the dengue virus is dengue type 2 virus and the virus is attenuated by a deletion of about 30 nucleotides from the 3' untranslated region of the dengue type 2 genome corresponding to the TL2 stem-loop structure between about nucleotides 10541-10570.

10. The nucleic acid chimera of claim 6, wherein the dengue virus is dengue type 3 virus and the virus is attenuated by a deletion of about 30 nucleotides from the 3' untranslated region of the dengue type 3 genome corresponding to the TL2 stem-loop structure between about nucleotides 10535-10565.

11. The nucleic acid chimera of claim 1, wherein the first nucleotide sequence encodes at least two structural proteins from a West Nile virus.

12. The nucleic acid chimera of claim 11, wherein the structural proteins are prM and E proteins.

13. The nucleic acid chimera of claim 12, wherein the dengue virus is dengue type 4 virus, wherein a cleavage site is utilized for joining a dengue virus capsid protein and a West Nile virus prM protein, and wherein the West Nile virus prM protein contains aspartic acid (Asp) at a position 3 amino acids downstream of the cleavage site and contains threonine (Thr) at a position 6 amino acids downstream of the cleavage site.

14. A virus chimera comprising one or more than one nucleic acid chimera of claim 1.

15. An immunogenic composition comprising one or more than one nucleic acid chimera of claim 1 or one or more than one virus chimera of claim 14 and a pharmaceutically acceptable carrier.

16. The composition of claim 15 for use in the induction of an immune response.

17. A method of inducing an immune response in a subject comprising administering an effective amount of the composition of claim 15 to the subject.

18. The method of claim 17 wherein the subject is a non-human primate.

19. The method of claim 17 wherein the subject is a human.

20. The method of claim 17 wherein the subject is a horse or a bird.

21. A vaccine composition comprising one or more than one nucleic acid chimera of claim 1 or one or more than one virus chimera of claim 14 and a pharmaceutically acceptable carrier.

caused by West Nile virus.

23. A method of preventing disease caused by West Nile virus in a subject comprising administering an effective amount of the composition of claim 21 to the subject.

24. The method of claim 23 wherein the subject is a non-human primate.

25. The method of claim 23 wherein the subject is a human.

26. The method of claim 23 wherein the subject is a horse or a bird.

27. An isolated nucleic acid probe or primer that selectively hybridizes with and possesses at least five nucleotides complementary to the nucleic acid or the complementary strand of the nucleic acid encoding the cleavage site that separates the capsid protein and the premembrane protein of the nucleic acid chimera of claim 12.

L1 ANSWER 9 OF 14 USPATFULL on STN
2005:11907 Development of mutations useful for attenuating dengue viruses and chimeric dengue viruses.
Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Hanley, Kathryn A., Bethesda, MD, UNITED STATES
Blaney, Joseph E., Frederick, MD, UNITED STATES
US 2005010043 A1 20050113
APPLICATION: US 2003-719547 A1 20031121 (10)
PRIORITY: US 2001-293049P 20010522 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A flavivirus having a phenotype in which the viral genome is modified by the introduction of a mutation, singly or in combination, taken from the group consisting of the mutations of any of Table 1-37, preferably Table 37.

2. The flavivirus of claim 1, further comprising the Δ30 mutation.

3. The flavivirus of claim 1, wherein the flavivirus is a dengue virus type 1.

4. The flavivirus of claim 1, wherein the flavivirus is a dengue virus type 2.

5. The flavivirus of claim 1, wherein the flavivirus is a dengue virus type 3.

6. The flavivirus of claim 1, wherein the flavivirus is a dengue virus type 4.

7. The flavivirus of claim 1, wherein the flavivirus is a chimeric virus.

8. The chimeric virus of claim 7 having a dengue 1 backbone.

9. The chimeric virus of claim 7 having a dengue 2 backbone.

10. The chimeric virus of claim 7 having a dengue 3 backbone.

11. The chimeric virus of claim 7 having a dengue 4 backbone.

12. The flavivirus of claim 1, wherein the phenotype is temperature sensitivity in Vero cells or the human liver cell line HuH-7.

13. The flavivirus of claim 1, wherein the phenotype is host-cell restriction in mosquito cells or the human liver cell line HuH-7.

14. The flavivirus of claim 1, wherein the phenotype is host-cell adaptation for improved replication in Vero cells.

15. The flavivirus of claim 1, wherein the phenotype is attenuation in mice.

16. A pharmaceutical composition comprising a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

17. A kit comprising a pharmaceutical composition according to claim 16 in a pack or dispenser device and instructions for administration.

18. A method of producing neutralizing antibodies against dengue virus comprising the administration of a therapeutically effective amount of a

vehicle and a flavivirus according to any of claims 1-15.

19. The method of claim 18, wherein administration is by subcutaneous, intradermal, or intramuscular injection.

20. A tetravalent vaccine comprising a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

21. An live attenuated vaccine comprising a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

22. The live attenuated vaccine of claim 21 in unit dosage form having from about 10^{2-10^9} plaque forming units (PFU)/ml.

23. An inactivated vaccine comprising a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

24. The inactivated vaccine of claim 23 in unit dosage form having from about 0.1 to 50 µg of E protein/ml.

25. A cDNA molecule encoding a flavivirus according to any of claims 1-15.

26. An RNA molecule encoding a flavivirus according to any of claims 1-15.

27. A method of preparing a flavivirus comprising (a) synthesizing full-length viral genomic RNA in vitro using a cDNA molecule that encodes a flavivirus according to any of claims 1-15; (b) transfecting cultured cells with the viral genomic RNA to produce virus; and (c) isolating the virus from the cultured cells.

28. A method of making a pharmaceutical composition comprising combining a pharmacologically acceptable vehicle and a flavivirus according to any of claims 1-15.

29. A method of identifying a mutation that restricts replication in human liver cells comprising (a) introducing mutations into a dengue virus genome to make mutant viruses; (b) screening the mutant viruses for a phenotype characterized by host-cell restriction in human liver cells; and (c) determining the genetic basis for the phenotype by direct sequence analysis of the virus genome.

30. A method of identifying a mutation that promotes growth in Vero cells comprising (a) introducing mutations into a dengue virus genome to make mutant viruses; (b) screening the mutant viruses for a phenotype characterized by host-cell adaptation for improved replication in Vero cells; and (c) determining the genetic basis for the phenotype by direct sequence analysis of the virus genome.

31. A method of assembling a menu of mutations for use in fine-tuning the attenuation and growth characteristics of recombinant dengue viruses comprising (a) introducing mutations into a dengue virus genome to make mutant viruses; (b) screening the mutant viruses for a phenotype characterized by temperature sensitivity in Vero cells or human liver cells, host cell restriction in mosquito cells or human liver cells, host-cell adaptation for improved replication in Vero cells, or attenuation in mice; (c) determining the genetic basis for the phenotype by direct sequence analysis of the virus genome; and (d) performing multiple iterations of steps (a)-(c), whereby a menu of mutations is assembled.

32. The method of any of claims 29-30 further comprising introducing the mutation identified by said method into a recombinant dengue virus, and characterizing the resulting phenotype.

```
=> e murphy brian r/in
E1          2     MURPHY BRIAN P/IN
E2          1     MURPHY BRIAN PATRICK/IN
E3         32 --> MURPHY BRIAN R/IN
E4          4     MURPHY BRIAN T/IN
E5          1     MURPHY BRIAN W/IN
E6          2     MURPHY BRIDGETTE/IN
E7          1     MURPHY BRITT/IN
E8          1     MURPHY BROOK CHANDLER/IN
E9          1     MURPHY BRUCE/IN
E10         3     MURPHY BRUCE D/IN
E11         1     MURPHY BRUCE E/IN
E12         5     MURPHY BRUCE L/IN
```

```
L2          32 "MURPHY BRIAN R"/IN

=> s l2 and dengue
         2809 DENGUE
L3           3 L2 AND DENGUE

=> d l3,cbib,1-3

L3   ANSWER 1 OF 3  USPATFULL on STN
2005:117619 Construction of West Nile virus and dengue virus chimeras for use
    in a live virus vaccine to prevent disease caused by West Nile virus.
    Pletnev, Alexander G., Rockville, MD, UNITED STATES
    Putnak, Joseph R., Silver Spring, MD, UNITED STATES
    Chanock, Robert M., Bethesda, MD, UNITED STATES
    Murphy, Brian R., Bethesda, MD, UNITED STATES
    Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
    Blaney, Joseph E. JR., Frederick, MD, UNITED STATES
    US 2005100886 A1 20050512
    APPLICATION: US 2004-871775  A1  20040618 (10)
    PRIORITY: US 2002-347281P  20020110 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 2 OF 3  USPATFULL on STN
2005:11907 Development of mutations useful for attenuating dengue viruses and
    chimeric dengue viruses.
    Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
    Murphy, Brian R., Bethesda, MD, UNITED STATES
    Hanley, Kathryn A., Bethesda, MD, UNITED STATES
    Blaney, Joseph E., Frederick, MD, UNITED STATES
    US 2005010043 A1 20050113
    APPLICATION: US 2003-719547  A1  20031121 (10)
    PRIORITY: US 2001-293049P  20010522 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3   ANSWER 3 OF 3  USPATFULL on STN
2002:280145 Use of recombinant parainfluenza viruses (PIVs) as vectors to
    protect against infection and disease caused by PIV and other human
    pathogens.
    Murphy, Brian R., Bethesda, MD, UNITED STATES
    Collins, Peter L., Rockville, MD, UNITED STATES
    Schmidt, Alexander C., Washington, DC, UNITED STATES
    Durbin, Anna P., Takoma Park, MD, UNITED STATES
    Skiadopoulos, Mario H., Potomac, MD, UNITED STATES
    Tao, Tao, Bethesda, MD, UNITED STATES
    US 2002155581 A1 20021024
    APPLICATION: US 2000-733692  A1  20001208 (9)
    PRIORITY: US 1997-47575P  19970523 (60)
    US 1997-59385P  19970919 (60)
    US 1999-170195P  19991210 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> e hanley kathryn a/in
E1           5     HANLEY KATHLEEN M/IN
E2           1     HANLEY KATHLEEN MARIE/IN
E3           1 --> HANLEY KATHRYN A/IN
E4           2     HANLEY KEITH J/IN
E5          11     HANLEY KENNETH J/IN
E6           1     HANLEY KENNETH JASON/IN
E7           2     HANLEY KEVIN/IN
E8           1     HANLEY KEVIN E/IN
E9           1     HANLEY KYLE P/IN
E10          1     HANLEY LITA/IN
E11          2     HANLEY LUKE/IN
E12          1     HANLEY MARCUS THOROLD/IN

=> s e3
L4           1 "HANLEY KATHRYN A"/IN

=> d his

     (FILE 'HOME' ENTERED AT 01:43:33 ON 10 JUL 2006)

     FILE 'USPATFULL' ENTERED AT 01:43:53 ON 10 JUL 2006
                E WHITEHEAD STEPHEN S/IN
L1          14 S E3
                E MURPHY BRIAN R/IN
L2          32 S E3
L3           3 S L2 AND DENGUE
```

```
L4            1 S E3

=> d l4,cbib,

L4   ANSWER 1 OF 1  USPATFULL on STN
2005:11907 Development of mutations useful for attenuating dengue viruses and
    chimeric dengue viruses.
    Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
    Murphy, Brian R., Bethesda, MD, UNITED STATES
    Hanley, Kathryn A., Bethesda, MD, UNITED STATES
    Blaney, Joseph E., Frederick, MD, UNITED STATES
    US 2005010043 A1 20050113
    APPLICATION: US 2003-719547  A1  20031121 (10)
    PRIORITY: US 2001-293049P  20010522 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> e blaney joseph e/in
E1            1     BLANEY JEFF/IN
E2            4     BLANEY JEFFREY M/IN
E3            1 --> BLANEY JOSEPH E/IN
E4            1     BLANEY JOSEPH E JR/IN
E5            1     BLANEY KEN FRANCIS/IN
E6            1     BLANEY KEVIN/IN
E7            1     BLANEY LAWRENCE F/IN
E8            1     BLANEY LORAN F/IN
E9            1     BLANEY LOREN F/IN
E10           1     BLANEY LYNETTE/IN
E11           1     BLANEY LYNNETTE D/IN
E12           1     BLANEY MARK A/IN

=> s e3-e4
              1 "BLANEY JOSEPH E"/IN
              1 "BLANEY JOSEPH E JR"/IN
L5            2 ("BLANEY JOSEPH E"/IN OR "BLANEY JOSEPH E JR"/IN)

=> s l5 not (l1 or l2)
L6            0 L5 NOT (L1 OR L2)

=> file wpids
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                               32.01      32.22

FILE 'WPIDS' ENTERED AT 01:48:19 ON 10 JUL 2006
COPYRIGHT (C) 2006 THE THOMSON CORPORATION

FILE LAST UPDATED:          6 JUL 2006      <20060706/UP>
MOST RECENT DERWENT UPDATE:     200643        <200643/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> FOR A COPY OF THE DERWENT WORLD PATENTS INDEX STN USER GUIDE,
    PLEASE VISIT:
 http://www.stn-international.de/training_center/patents/stn_guide.pdf <

>>> FOR DETAILS OF THE PATENTS COVERED IN CURRENT UPDATES, SEE
http://scientific.thomson.com/support/patents/coverage/latestupdates/

>>> PLEASE BE AWARE OF THE NEW IPC REFORM IN 2006, SEE
http://www.stn-international.de/stndatabases/details/ipc_reform.html and
http://scientific.thomson.com/media/scpdf/ipcrdwpi.pdf <<<

>>> FOR FURTHER DETAILS ON THE FORTHCOMING DERWENT WORLD PATENTS
    INDEX ENHANCEMENTS PLEASE VISIT:
http://www.stn-international.de/stndatabases/details/dwpi_r.html <<<

=> e whitehead s s/in
E1            2     WHITEHEAD S J/IN
E2           33     WHITEHEAD S P/IN
E3            9 --> WHITEHEAD S S/IN
E4            7     WHITEHEAD T/IN
E5            3     WHITEHEAD T A/IN
E6            2     WHITEHEAD T D/IN
E7            1     WHITEHEAD T F M/IN
E8            6     WHITEHEAD T P/IN
E9            3     WHITEHEAD T W/IN
E10           2     WHITEHEAD V/IN
E11           1     WHITEHEAD V B/IN
E12          19     WHITEHEAD V E/IN

=> s e3
```

```
=> d his

     (FILE 'HOME' ENTERED AT 01:43:33 ON 10 JUL 2006)

     FILE 'USPATFULL' ENTERED AT 01:43:53 ON 10 JUL 2006
                E WHITEHEAD STEPHEN S/IN
L1            14 S E3
                E MURPHY BRIAN R/IN
L2            32 S E3
L3             3 S L2 AND DENGUE
                E HANLEY KATHRYN A/IN
L4             1 S E3
                E BLANEY JOSEPH E/IN
L5             2 S E3-E4
L6             0 S L5 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 01:48:19 ON 10 JUL 2006
                E WHITEHEAD S S/IN
L7             9 S E3

=> s l7 not l1 or l2
               0 "WHITEHEAD STEPHEN S"/IN
               0 "MURPHY BRIAN R"/IN
L8             9 L7 NOT L1 OR L2

=> del l8
DELETE L8? (Y)/N:y

=> s l7 not (l1 or l2)
               0 "WHITEHEAD STEPHEN S"/IN
               0 "MURPHY BRIAN R"/IN
L8             9 L7 NOT (L1 OR L2)

=> d l8,ti,1-9

L8   ANSWER 1 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New tetravalent vaccine containing a common nucleotide deletion in the 3'
     untranslated region of dengue types 1, 2, 3, and 4, useful for preventing
     of disease in humans caused by dengue virus, or for inducing immune
     response.

L8   ANSWER 2 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Novel nucleic acid chimera comprising nucleic acids encoding structural
     protein from West Nile virus and non-structural proteins from wild-type
     strain of dengue virus useful for producing live West Nile virus vaccines.

L8   ANSWER 3 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   New mutated flavivirus, useful for fine tuning the attenuation and growth
     characteristics of dengue virus vaccines for the prevention and/or
     treatment of dengue virus infection.

L8   ANSWER 4 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   An isolated infectious recombinant respiratory syncytial virus (RSV)
     having one or more shifted RSV gene(s) or genome segment(s) within the
     recombinant genome or antigenome, useful as an attenuated vaccine against
     RSV strains.

L8   ANSWER 5 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Isolated chimeric human-bovine respiratory syncytial virus (RSV), useful
     in an attenuated vaccine to elicits an immune response against either or
     both human RSV A or RSV B.

L8   ANSWER 6 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Recombinant respiratory syncytial virus (RSV) incorporating a heterologous
     polynucleotide encoding an immune modulatory molecule is used as a vaccine
     to provide an immune response to RSV.

L8   ANSWER 7 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Infectious chimeric respiratory syncytial virus (RSV) produced from cloned
     nucleotide sequences, useful as a vaccine against diseases caused by the
     virus, such as pneumoniae and bronchiolitis.

L8   ANSWER 8 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Attenuated respiratory syncytial virus vaccines - useful to protect
     individuals against RSV infection.

L8   ANSWER 9 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
TI   Infectious respiratory syncytial virus particles - useful for treatment of
     RSV or gene therapy of upper respiratory tract diseases.
```

'CBIB' IS NOT A VALID FORMAT FOR FILE 'WPIDS'
'CLM' IS NOT A VALID FORMAT FOR FILE 'WPIDS'

The following are valid formats:

TRI	SAM	Short Information (Syn.: TRIAL,SAMPLE)
STR		DERWENT Chemical Resource Structure
HITSTR		HITSTRUCTURES
BIB		Bibliographic Data
BRIEFG.H		Brief Contents of Document with GI.H
BRIEFG		Brief Contents of Document with GI
BRIEF		Brief Contents of Document
IBRIEFG.H		Brief Contents of Document with GI.H, Indented Version
IBRIEFG		Brief Contents of Document with GI, Indented Version
IBRIEF		Brief Contents of Document, Indented Version
MAXG		All Data with GIS and GI.H
MAX		All Data
ALLG.H		All Data Except ABEQ, CMC, and PLC with GI.H
ALLG		All Data Except ABEQ, CMC, and PLC with GI
ALL		All Data Except ABEQ, CMC, and PLC
FULL		All Data Except ABEQ, CMC, and PLC plus TECH and PRIO
FULLG		All Data Except ABEQ, CMC, and PLC with GI plus TECH and PRIO
DALL		Delimited ALL Format
BASIC		Basic Patent Information
STD		Default
IDE		Structure File Default
IALLG.H		Indented Version of ALL Format with GI.H
IALLG		Indented Version of ALL Format with GI
IALL		Indented Version of ALL Format
IFULL		Indented Version of FULL Format
IFULLG		Indented Version of FULLG Format
ISTD		Indented Version of STD Format
IBIB		Indented Version of BIB Format
ABS		All Abstracts
CODE	IND	Manual-, Plasdoc-, and Chemical Code plus Keywords
SUM		Title and Novelty

AB		Abstract (Basic)
ABEQ		Abstract, Equivalent
ADT		Application Details
ADT.B		Application Details Basic
AI	AP	Application Information
AI.B		Application Information Basic
AN		Accession Number
AN.S		DERWENT Chemistry Resource Accession Number, DCR Segment
APPS		Application Number Group
AW		Additional Words
CC		Classification Code (Substance Descriptor
CMC		Chemical Code
CMT		Comment
CN		Chemical Name
CN.P		Chemical Name Preferred
CN.S		Systematic Chemical Name
CR	XR	Cross Reference
CYC		Country Count
DAN		DERWENT Accession Number List
DC		DERWENT Class
DCN		DERWENT Compound Number
DCR		DERWENT Chemistry Resource Accession Number
DCRE		DERWENT Chemistry Resource Number
DCSE		DERWENT Chemistry Resource Number, DCR Segment
DN		Document Number CPI and Non CPI
DNC		Document Number CPI
DNN		Document Number Non CPI
DRN		DERWENT Registry Number
DS		Designated States
ED		Entry Date
EDCR		Entry Date DERWENT Chemistry Resource
FA		Field Availability
FAS		Field Availability Supplementary Data
FAM		Patent Family
FDT		Filing Details
FG	AM	Fragment Code
FS		File Segment
IC		International Patent Classification
GI		Graphical Information
GI.H		Graphical Information, High Resolution
GIS		Graphical Information Size
ICA		IPC, Additional (Supplementary)
ICI		IPC, Index (Complementary)
ICM		IPC, Main

```
IN    AU    Inventor
IPC         International Patent Classification
KS          Plasdoc Key Serials
KW          Keyword Indexing, Including DERWENT Chemistry Resource Numbers, DWPI Segment
M0          Chemical Code (Pre 1970)
M1-6        Chemical Codes
MC          Manual Code
MF          Molecular Formula
MW          Molecular Weight
NOV         Novelty
PA    CS    Patent Assignee
PATS        Patent Number Group
PI          Patent Information
PI.B  PN.B  Patent Information Basic
PIA         Patent Information Abbreviated
PIA.B       Patent Information Abbreviated Basic
PLC         Plasdoc Codes
PLE         Enhanced Plasdoc Codes
PN          Patent Number
PNC         Patent Number Count
PRAI  PRN   Priority Information
PRIO        Prior Art
REP  RPN RE Reference Patent Information
RIN         Ring Index Number
SDCN        Structure Segment DERWENT Compound Number
SDRN        Structure Segment DERWENT Registry Number
SMF         Standardized Molecular Formula
SRIN        Structure Segment Ring Index Number
SY          Synonym Name
TECH        Technology Focus
TI          Title
TT          Title Terms
UP          Update Date
UPA         Update Date Plasdoc Code
UPAB        Update Date Abstract
UPB         Update Date Chemical Code
UPCR        Update Date DERWENT Chemistry Resource
UPKW        Update Date Keyword Indexing
UPP         Update Date Patent
UPS         Update Date SDI
UPTX        Update New Content Abstract Fields
UPWX        Update Date WPI Cross Reference
ENTER DISPLAY FORMAT (STD):bib

L8   ANSWER 3 OF 9  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   2003-120809 [11]   WPIDS
DNN  N2003-096140       DNC C2003-031374
TI   New mutated flavivirus, useful for fine tuning the attenuation and growth
     characteristics of dengue virus vaccines for the prevention and/or
     treatment of dengue virus infection.
DC   B04 D16 S03
IN   HANLEY, K A; MURPHY, B R; WHITEHEAD, S S; BLANEY, J E
PA   (BLAN-I) BLANEY J E; (USSH) US DEPT HEALTH & HUMAN SERVICES; (HANL-I)
     HANLEY K A; (MURP-I) MURPHY B R; (WHIT-I) WHITEHEAD S S
CYC  101
PI   WO 2002095075   A1 20021128 (200311)* EN  246
        RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
            NL OA PT SD SE SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
            RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM
            ZW
     BR 2002009943   A  20040330 (200424)
     EP 1402075      A1 20040331 (200424)  EN
         R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
            RO SE SI TR
     AU 2002312011   A1 20021203 (200452)
     US 2005010043   A1 20050113 (200506)
     IN 2003002184   P1 20050603 (200602)  EN
ADT  WO 2002095075 A1 WO 2002-US16308 20020522; BR 2002009943 A BR 2002-9943
     20020522, WO 2002-US16308 20020522; EP 1402075 A1 EP 2002-739358 20020522,
     WO 2002-US16308 20020522; AU 2002312011 A1 AU 2002-312011 20020522; US
     2005010043 A1 Provisional US 2001-293049P 20010522, Cont of WO
     2002-US16308 20020522, US 2003-719547 20031121; IN 2003002184 P1 WO
     2002-US16308 20020522, IN 2003-DN2184 20031215
FDT  BR 2002009943 A Based on WO 2002095075; EP 1402075 A1 Based on WO
     2002095075; AU 2002312011 A1 Based on WO 2002095075
PRAI US 2001-293049P      20010522; US 2003-719547       20031121
```

```
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    16.63      48.85

FILE 'MEDLINE' ENTERED AT 01:50:04 ON 10 JUL 2006

 FILE LAST UPDATED: 8 JUL 2006 (20060708/UP).  FILE COVERS 1950 TO DATE.

 On December 11, 2005, the 2006 MeSH terms were loaded.

 The MEDLINE reload for 2006 is now (26 Feb.) available.  For details
 on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
 See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

 OLDMEDLINE is covered back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2006 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> e whitehead s s/au
E1          15     WHITEHEAD S M/AU
E2           2     WHITEHEAD S P/AU
E3          31 --> WHITEHEAD S S/AU
E4           1     WHITEHEAD S W/AU
E5           3     WHITEHEAD SAFFRON A/AU
E6           5     WHITEHEAD SALLY/AU
E7           6     WHITEHEAD SARA/AU
E8           2     WHITEHEAD SARA J/AU
E9           2     WHITEHEAD SARAH E/AU
E10          2     WHITEHEAD SHARON/AU
E11          1     WHITEHEAD SHAUN A/AU
E12          1     WHITEHEAD SHAWN/AU

=> e whitehead stephen/au
E1           1     WHITEHEAD SIOBHAN/AU
E2           1     WHITEHEAD STACEY/AU
E3           0 --> WHITEHEAD STEPHEN/AU
E4          13     WHITEHEAD STEPHEN S/AU
E5           9     WHITEHEAD STEVE/AU
E6           1     WHITEHEAD STEVEN M/AU
E7           2     WHITEHEAD SUE/AU
E8           1     WHITEHEAD SUSAN/AU
E9           1     WHITEHEAD SUSAN F/AU
E10         45     WHITEHEAD T/AU
E11          2     WHITEHEAD T C/AU
E12          3     WHITEHEAD T D/AU

=> s e4-e5
            13 "WHITEHEAD STEPHEN S"/AU
             9 "WHITEHEAD STEVE"/AU
L9          22 ("WHITEHEAD STEPHEN S"/AU OR "WHITEHEAD STEVE"/AU)

=> d l9,cbib,ab,1-22

L9   ANSWER 1 OF 22     MEDLINE on STN
2006166217.    PubMed ID: 16553547.   Development of a live attenuated dengue
     virus vaccine using reverse genetics. Blaney Joseph E Jr; Durbin Anna P;
     Murphy Brian R; Whitehead Stephen S. (Laboratory of Infectious Diseases,
     National Institute of Allergy and Infectious Diseases, National Institutes
     of Health, LID, Bethesda, Maryland 20892-8133, USA..
     jblaney@niaid.nih.gov) . Viral immunology, (2006 Spring) Vol. 19, No. 1,
     pp. 10-32.  Journal code: 8801552. ISSN: 0882-8245. Pub. country: United
     States. Language: English.
AB   There are four serotypes of dengue (DEN1-DEN4) virus that are endemic in
     most areas of Southeast Asia, Central and South America, and other
     subtropical regions.  The number of cases of severe disease associated
     with DEN virus infection is growing because of the continued spread of the
     mosquito vector, Aedes aegypti, which transmits the virus to humans.
     Infection with DEN virus can result in an asymptomatic infection, a
     febrile illness called dengue fever (DF), and the very severe disease
     called dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS).
     Currently, a licensed vaccine is not available.  However, a tetravalent
     vaccine is urgently needed to prevent DF and DHF/DSS, the latter of which
     occurs predominantly in partially immune individuals.  A live attenuated,
```

genetics that is able to provide immunity to each of the four serotypes of DEN. Attenuation has been achieved by generating recombinant DEN (rDEN) viruses which are modified by deletion or, alternatively, by antigenic chimerization between two related DEN viruses using the following two strategies: 1) introduction of an attenuating 30 nucleotide deletion (Delta30) mutation into the 3' untranslated region of DEN1 and DEN4; and 2) replacement of structural proteins of the attenuated rDEN4Delta30 vaccine candidate with those from DEN2 or DEN3. Attenuation of the four monovalent vaccine candidates has been achieved for rhesus monkeys or humans and an immunogenic tetravalent vaccine candidate has been formulated. The level of attenuation of each dengue vaccine component can be increased, if needed, by introduction of additional attenuating mutations that have been well characterized.

L9 ANSWER 2 OF 22 MEDLINE on STN

2005261290. PubMed ID: 15902081. A rational response to Taser strikes. **Whitehead Steve**. (Mountain View Fire Protection District, Mountain View, USA.. swhitehead@mountainviewfire.org) . JEMS : a journal of emergency medical services, (2005 May) Vol. 30, No. 5, pp. 56-66. Journal code: 8102138. ISSN: 0197-2510. Pub. country: United States. Language: English.

L9 ANSWER 3 OF 22 MEDLINE on STN

2005205458. PubMed ID: 15839544. Memorable patients and the lessons they teach. **Whitehead Steve**; Bledsoe Bryan; Dalton Alice; Werfel Paul; Taigman Mike. (Mountain View (CO) Fire Protection District, USA.. eonflux@aol.com) . Emergency medical services, (2005 Mar) Vol. 34, No. 3, pp. 95-7. Journal code: 0431735. ISSN: 0094-6575. Pub. country: United States. Language: English.

L9 ANSWER 4 OF 22 MEDLINE on STN

2005193888. PubMed ID: 15827166. Recombinant, live-attenuated tetravalent dengue virus vaccine formulations induce a balanced, broad, and protective neutralizing antibody response against each of the four serotypes in rhesus monkeys. Blaney Joseph E Jr; Matro Jennifer M; Murphy Brian R; **Whitehead Stephen S**. (Laboratory of Infectious Diseases, NIH, NIAID, LID Twinbrook III, Room 3W-13, 12735 Twinbrook Parkway, MSC 8133, Bethesda, MD 20892-8133, USA.. jblaney@niaid.nih.gov) . Journal of virology, (2005 May) Vol. 79, No. 9, pp. 5516-28. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Three tetravalent vaccine (TV) formulations of previously described monovalent dengue (DEN) virus vaccine candidates were compared to a tetravalent formulation of wild-type DEN viruses (T-wt) for replication in SCID mice transplanted with human liver cells (SCID-HuH-7) or for replication and immunogenicity in rhesus monkeys. TV-1 consists of recombinant DEN1, -2, -3, and -4, each with a 30-nucleotide deletion in the 3' untranslated region (Delta30). TV-2 consists of rDEN1Delta30, rDEN4Delta30, and two antigenic chimeric viruses, rDEN2/4Delta30 and rDEN3/4Delta30, both also bearing the Delta30 mutation. TV-3 consists of rDEN1Delta30, rDEN2Delta30, rDEN4Delta30, and a 10-fold higher dose of rDEN3/4Delta30. TV-1 and TV-2 were attenuated in SCID-HuH-7 mice with minimal interference in replication among the virus components. TV-1, -2, and -3 were attenuated in rhesus monkeys as measured by duration and peak of viremia. Each monkey immunized with TV-1 and TV-3 seroconverted to the four DEN components by day 28 with neutralization titers ranging from 1:52 to 1:273 and 1:59 to 1:144 for TV-1 and TV-3, respectively. TV-2 induced low antibody titers to DEN2 and DEN3, but a booster immunization after 4 months increased the neutralizing antibody titers to greater than 1:100 against each serotype and elicited broad neutralizing activity against 19 of 20 DEN subtypes. A single dose of TV-2 induced protection against wild-type DEN1, DEN3, and DEN4 challenge, but not DEN2. However, two doses of TV-2 or TV-3 induced protection against DEN2 challenge. Two tetravalent formulations, TV-2 and TV-3, possess properties of a successful DEN vaccine and can be considered for evaluation in clinical trials.

L9 ANSWER 5 OF 22 MEDLINE on STN

2005058909. PubMed ID: 15688284. rDEN4delta30, a live attenuated dengue virus type 4 vaccine candidate, is safe, immunogenic, and highly infectious in healthy adult volunteers. Durbin Anna P; **Whitehead Stephen S**; McArthur Julie; Perreault John R; Blaney Joseph E Jr; Thumar Bhavin; Murphy Brian R; Karron Ruth A. (Center for Immunization Research, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205, USA.. adurbin@jhsph.edu) . The Journal of infectious diseases, (2005 Mar 1) Vol. 191, No. 5, pp. 710-8. Electronic Publication: 2005-01-27. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB BACKGROUND: The live attenuated dengue virus type 4 (DEN-4) vaccine candidate virus rDEN4 Delta 30 was previously found to be safe and immunogenic at a dose of 10(5) plaque-forming units (pfu). METHODS: In a follow-up placebo-controlled phase 2 clinical trial, rDEN4 Delta 30 was administered as a single inoculation to 3 separate dose cohorts (10(3) pfu, 10(2) pfu, or 10(1) pfu), for further evaluation. Each dose cohort

monitored closely for adverse events, and serum was collected on study days 28 and 42 for determination of neutralizing antibody titer. RESULTS: The vaccine was well tolerated at all doses studied. The most common adverse events observed were a transient asymptomatic rash in >50% of vaccinees and a mild neutropenia in approximately 20% of vaccinees. No vaccinee developed a dengue-like illness. The vaccine was highly infectious and immunogenic, with 95%-100% of vaccinees in each dose cohort developing a >/=4-fold increase in titers of serum neutralizing antibodies against DEN-4. CONCLUSIONS: The rDEN4 Delta 30 vaccine is safe and induced an antibody response that was broadly neutralizing against genotypically diverse DEN-4 viruses. It is a promising vaccine candidate for inclusion in a tetravalent dengue vaccine formulation.

L9 ANSWER 6 OF 22 MEDLINE on STN

2005016122. PubMed ID: 15642976. Genetically modified, live attenuated dengue virus type 3 vaccine candidates. Blaney Joseph E Jr; Hanson Christopher T; Firestone Cai-Yen; Hanley Kathryn A; Murphy Brian R; **Whitehead Stephen S**. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-8007, USA.. jblaney@niaid.nih.gov) . The American journal of tropical medicine and hygiene, (2004 Dec) Vol. 71, No. 6, pp. 811-21. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB Three novel recombinant dengue type 3 (DEN3) virus vaccine candidates have been generated from a DEN3 virus isolated from a mild outbreak of dengue fever in the Sleman area of central Java in Indonesia in 1978. Antigenic chimeric viruses were prepared by replacing the membrane precursor and envelope (ME) proteins of recombinant DEN4 (rDEN4) virus with those from DEN3 Sleman/78 in the presence (rDEN3/4Delta30(ME)) and the absence (rDEN3/4(ME)) of the Delta30 mutation, a previously described 30-nucleotide deletion in the 3' untranslated region. In addition, a full-length infectious cDNA clone was generated from the DEN3 isolate and used to produce rDEN3 virus and the vaccine candidate rDEN3Delta30. The chimeric viruses rDEN3/4(ME) and rDEN3/4Delta30(ME) appear to be acceptable vaccine candidates since they were restricted in replication in severe combined immune deficiency mice transplanted with human hepatoma cells, in rhesus monkeys, and in Aedes and Toxorynchites mosquitoes, and each was protective in rhesus monkeys against DEN3 virus challenge. The rDEN3/4(ME) and rDEN3/4Delta30(ME) viruses were comparable in all parameters evaluated, indicating that antigenic chimerization resulted in the observed high level of attenuation. Surprisingly, rDEN3Delta30 was not attenuated in any model tested when compared with wild-type rDEN3 and therefore, is not a vaccine candidate at present. Thus, the rDEN3/4(ME) and rDEN3/4Delta30(ME) antigenic chimeric viruses can be considered for evaluation in humans and for inclusion in a tetravalent dengue vaccine.

L9 ANSWER 7 OF 22 MEDLINE on STN

2004577234. PubMed ID: 15553540. Views from the field. EMS Magazine's Third Annual Emerging Leaders in EMS Forum. Lanier Jim; McKenna Kimberly; Olsen Jonathan A; **Whitehead Steve**. Emergency medical services, (2004 Oct) Vol. 33, No. 10, pp. 148-54. Journal code: 0431735. ISSN: 0094-6575. Pub. country: United States. Language: English.

L9 ANSWER 8 OF 22 MEDLINE on STN

2004544655. PubMed ID: 15461822. Vaccine candidates derived from a novel infectious cDNA clone of an American genotype dengue virus type 2. Blaney Joseph E Jr; Hanson Christopher T; Hanley Kathryn A; Murphy Brian R; **Whitehead Stephen S**. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892 USA.. jblaney@niaid.nih.gov) . BMC infectious diseases [electronic resource], (2004 Oct 4) Vol. 4, pp. 39. Electronic Publication: 2004-10-04. Journal code: 100968551. E-ISSN: 1471-2334. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: A dengue virus type 2 (DEN-2 Tonga/74) isolated from a 1974 epidemic was characterized by mild illness and belongs to the American genotype of DEN-2 viruses. To prepare a vaccine candidate, a previously described 30 nucleotide deletion (Delta30) in the 3' untranslated region of DEN-4 has been engineered into the DEN-2 isolate. METHODS: A full-length cDNA clone was generated from the DEN-2 virus and used to produce recombinant DEN-2 (rDEN-2) and rDEN2Delta30. Viruses were evaluated for replication in SCID mice transplanted with human hepatoma cells (SCID-HuH-7 mice), in mosquitoes, and in rhesus monkeys. Neutralizing antibody induction and protective efficacy were also assessed in rhesus monkeys. RESULTS: The rDEN2Delta30 virus was ten-fold reduced in replication in SCID-HuH-7 mice when compared to the parent virus. The rDEN-2 viruses were not infectious for Aedes mosquitoes, but both readily infected Toxorynchites mosquitoes. In rhesus monkeys, rDEN2Delta30 appeared to be slightly attenuated when compared to the parent virus as measured by duration and peak of viremia and neutralizing antibody induction. A derivative of rDEN2Delta30, designated rDEN2Delta30-4995, was generated by incorporation of a point mutation previously identified in the NS3 gene of DEN-4 and was found to be more attenuated than

rDEN2Delta30-4995 viruses can be considered for evaluation in humans and for inclusion in a tetravalent dengue vaccine.

L9 ANSWER 9 OF 22 MEDLINE on STN
2004426988. PubMed ID: 15326447. Trailblazing: the latest innovations in emergency vehicle lights & sirens. **Whitehead Steve**. (Pridemark Paramedic Services, Arvada, Colo., USA.) JEMS : a journal of emergency medical services, (2004 Aug) Vol. 29, No. 8, pp. 44-5. Journal code: 8102138. ISSN: 0197-2510. Pub. country: United States. Language: English.

L9 ANSWER 10 OF 22 MEDLINE on STN
2004405248. PubMed ID: 15308370. Introduction of mutations into the non-structural genes or 3' untranslated region of an attenuated dengue virus type 4 vaccine candidate further decreases replication in rhesus monkeys while retaining protective immunity. Hanley Kathryn A; Manlucu Luella R; Manipon Gracielle G; Hanson Christopher T; **Whitehead Stephen S**; Murphy Brian R; Blaney Joseph E Jr. (Laboratory of Infectious Diseases (LID), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Building 50, Room 6515, 50 South Drive, MSC 8007, Bethesda, MD 20892-8007, USA.) Vaccine, (2004 Sep 3) Vol. 22, No. 25-26, pp. 3440-8. Journal code: 8406899. ISSN: 0264-410X. Pub. country: Netherlands. Language: English.
AB A dengue virus vaccine candidate, rDEN4Delta30, has been previously reported to be safe and immunogenic in humans, but a subset of vaccinees developed asymptomatic rash, elevation of liver enzymes and/or mild neutropenia. In the current study, mutations that had previously been shown to reduce replication of DEN4 virus in suckling mice and/or in SCID mice engrafted with human liver cells (SCID-HuH-7 mice) were introduced into rDEN4Delta30 in an attempt to further attenuate this virus. Three of the five resulting modified rDEN4Delta30 viruses showed decreased replication in SCID-HuH-7 mice relative to rDEN4Delta30. Moreover, in rhesus monkeys, two of the modified rDEN4Delta30 viruses showed a decrease in replication relative to rDEN4Delta30 while generating levels of neutralizing antibody similar to rDEN4Delta30 virus. All of the modified rDEN4Delta30 viruses completely protected immunized rhesus monkeys from challenge with wild-type DEN4 virus. Based on their attenuation for both human liver cells and rhesus monkeys, two of the modified rDEN4Delta30 vaccine candidates are currently being prepared for use in clinical trials. The application of these attenuating mutations to flavivirus vaccine development is discussed.

L9 ANSWER 11 OF 22 MEDLINE on STN
2004398764. PubMed ID: 15302187. Arguments for live flavivirus vaccines. Murphy Brian R; Blaney Joseph E Jr; **Whitehead Stephen S**. Lancet, (Aug 7-13 2004) Vol. 364, No. 9433, pp. 499-500. Journal code: 2985213R. E-ISSN: 1474-547X. Pub. country: England: United Kingdom. Language: English.

L9 ANSWER 12 OF 22 MEDLINE on STN
2004162524. PubMed ID: 15055075. Blood on tap. Part 2: An ethical dilemma in emergency research. **Whitehead Steve**. (Pridemark Paramedic Services, Arvada, CO, USA.) Emergency medical services, (2004 Mar) Vol. 33, No. 3, pp. 83-6. Journal code: 0431735. ISSN: 0094-6575.
Report No.: KIE-124301; NRCBL-VF 18.3. Pub. country: United States. Language: English.

L9 ANSWER 13 OF 22 MEDLINE on STN
2004105056. PubMed ID: 14994671. Blood on tap. Part 1. History in the making. **Whitehead Steve**. (Pridemark Paramedic Services, Arvada, CO, USA.. eonflux@aol.com) . Emergency medical services, (2004 Feb) Vol. 33, No. 2, pp. 41-8. Journal code: 0431735. ISSN: 0094-6575. Pub. country: United States. Language: English.
AB Emergency services around the United States are about to become part of the front lines in the race to bring to market the first blood substitute with oxygen-carrying capabilities. Take a look inside the high-tech world of biopharmaceuticals and the innovative pioneers who are chasing after a scientific holy grail; a synthetic substitute for blood. In the process, they have made and lost fortunes, advanced our understanding of the nature of blood and launched one of the biggest ethical controversies in modern medical history. As phase three clinical trials move to the prehospital arena, biotechnology firms are staking everything on the notion that they're about to change the way we treat trauma patients. They may be right.

L9 ANSWER 14 OF 22 MEDLINE on STN
2003446256. PubMed ID: 14505914. Mutations which enhance the replication of dengue virus type 4 and an antigenic chimeric dengue virus type 2/4 vaccine candidate in Vero cells. Blaney Joseph E Jr; Manipon Gracielle G; Firestone Cai Yen; Johnson Daniel H; Hanson Christopher T; Murphy Brian R; **Whitehead Stephen S**. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 50, Room 6515, 50 South Drive, MSC 8007, Bethesda, MD

Journal code: 8406899. ISSN: 0264-410X. Pub. country: Netherlands. Language: English.

AB Mutations which increase the replication of dengue viruses in cell culture would greatly facilitate the manufacture of both a live attenuated or inactivated dengue virus vaccine. We have identified eight missense mutations in dengue virus type 4 (DEN4) that increase the plaque size and kinetics of replication of recombinant DEN4 virus in Vero cells. DEN4 viruses bearing these Vero cell adaptation mutations were also evaluated for the level of replication in the brains of mice. Two of these eight recombinant viruses expressing distinct mutations in NS3 were both restricted in replication in the brains of suckling mice. In contrast, six recombinant viruses, each encoding individual mutations in NS4B (five) or in NS5 (one), were not attenuated in mouse brain. Recombinant viruses encoding various combinations of these Vero cell adaptation mutations did not demonstrate enhanced replication in Vero cells over that exhibited by the single mutations. Finally, addition of a subset of the above non-attenuating, adaptation mutations to a DEN2/4 chimeric vaccine candidate was found to increase the virus yield in Vero cells by up to 500-fold. The importance of these Vero cell adaptation mutations in flavivirus vaccine design and development is discussed.

L9 ANSWER 15 OF 22 MEDLINE on STN

2003446255. PubMed ID: 14505913. Substitution of the structural genes of dengue virus type 4 with those of type 2 results in chimeric vaccine candidates which are attenuated for mosquitoes, mice, and rhesus monkeys. **Whitehead Stephen S**; Hanley Kathryn A; Blaney Joseph E Jr; Gilmore Lara E; Elkins William R; Murphy Brian R. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Room 6515, Building 50, 50 South Drive, Bethesda, MD 20892-8007, USA.. swhitehead@niaid.nih.gov) . Vaccine, (2003 Oct 1) Vol. 21, No. 27-30, pp. 4307-16. Journal code: 8406899. ISSN: 0264-410X. Pub. country: Netherlands. Language: English.

AB Antigenic chimeric viruses in which the structural genes of dengue virus type 4 (DEN4) have been replaced with those derived from dengue virus type 2 (DEN2) have been created and evaluated as a first step in generating a live attenuated tetravalent dengue virus vaccine. Specifically, the capsid, membrane precursor, and envelope (CME) or the membrane precursor and envelope (ME) gene regions of DEN2 were substituted for the corresponding genes of wild-type rDEN4 or vaccine candidate rDEN4delta30 which contains a 30 nucleotide deletion in the 3' untranslated region. The two DEN2/4 chimeric viruses lacking the delta 30 mutation were highly attenuated in tumor-bearing SCID-HuH-7 mice, mosquitoes, and rhesus monkeys, indicating chimerization with either the CME or ME regions lead to attenuation. In mosquitoes and SCID-HuH-7 mice, addition of the delta 30 mutation to the chimeric viruses resulted in comparable or only slightly increased levels of attenuation. In rhesus monkeys, addition of the delta 30 mutation rendered the CME chimeric virus non-infectious, indicating that the attenuation resulting from chimerization and the delta 30 mutation were additive for these animals. In contrast, the attenuation in rhesus monkeys of ME chimeric virus was not significantly modified by the addition of the delta 30 mutation. The satisfactory level of attenuation and immunogenicity achieved by the ME containing DEN2/4delta 30 chimeric virus, as well as its very low infectivity for mosquitoes, make it a vaccine candidate suitable for evaluation in phase I clinical trials.

L9 ANSWER 16 OF 22 MEDLINE on STN

2003358821. PubMed ID: 12890635. A trade-off in replication in mosquito versus mammalian systems conferred by a point mutation in the NS4B protein of dengue virus type 4. Hanley Kathryn A; Manlucu Luella R; Gilmore Lara E; Blaney Joseph E Jr; Hanson Christopher T; Murphy Brian R; **Whitehead Stephen S**. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.. khanley@niaid.nih.gov) . Virology, (2003 Jul 20) Vol. 312, No. 1, pp. 222-32. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB An acceptable live-attenuated dengue virus vaccine candidate should have low potential for transmission by mosquitoes. We have identified and characterized a mutation in dengue virus type 4 (DEN4) that decreases the ability of the virus to infect mosquitoes. A panel of 1248 mutagenized virus clones generated previously by chemical mutagenesis was screened for decreased replication in mosquito C6/36 cells but efficient replication in simian Vero cells. One virus met these criteria and contained a single coding mutation: a C-to-U mutation at nucleotide 7129 resulting in a Pro-to-Leu change in amino acid 101 of the nonstructural 4B gene (NS4B P101L). This mutation results in decreased replication in C6/36 cells relative to wild-type DEN4, decreased infectivity for mosquitoes, enhanced replication in Vero and human HuH-7 cells, and enhanced replication in SCID mice implanted with HuH-7 cells (SCID-HuH-7 mice). A recombinant DEN4 virus (rDEN4) bearing this mutation exhibited the same set of phenotypes. Addition of the NS4B P101L mutation to rDEN4 bearing a 30 nucleotide deletion (Delta30) decreased the ability of the double-mutant

SCID-HuH-7 mice. Although the NS4B P101L mutation decreases infectivity of DEN4 for mosquitoes, its ability to enhance replication in SCID-HuH-7 mice suggests that it might not be advantageous to include this specific mutation in an rDEN4 vaccine. The opposing effects of the NS4B P101L mutation in mosquito and vertebrate systems suggest that the NS4B protein is involved in maintaining the balance between efficient replication in the mosquito vector and the human host.

L9 ANSWER 17 OF 22 MEDLINE on STN
2003312913. PubMed ID: 12841034. Extreme response. Flying with Air Rescue Five. **Whitehead Steve**; Forster Jeff. (Pridemark Paramedic Services, Arvada, CO, USA.) Emergency medical services, (2003 Jun) Vol. 32, No. 6, pp. 43-6, 48, 50 passim. Journal code: 0431735. ISSN: 0094-6575. Pub. country: United States. Language: English.

L9 ANSWER 18 OF 22 MEDLINE on STN
2003158556. PubMed ID: 12674579. The coming capnography wave. **Whitehead Steve**. Emergency medical services, (2003 Mar) Vol. 32, No. 3, pp. 89-90, 101. Journal code: 0431735. ISSN: 0094-6575. Pub. country: United States. Language: English.

L9 ANSWER 19 OF 22 MEDLINE on STN
2003047319. PubMed ID: 12556772. The capnography revolution begins. **Whitehead Steve**. JEMS : a journal of emergency medical services, (2003 Jan) Vol. 28, No. 1, pp. 130. Journal code: 8102138. ISSN: 0197-2510. Pub. country: United States. Language: English.

L9 ANSWER 20 OF 22 MEDLINE on STN
2003008679. PubMed ID: 12502885. A live, attenuated dengue virus type 1 vaccine candidate with a 30-nucleotide deletion in the 3' untranslated region is highly attenuated and immunogenic in monkeys. **Whitehead Stephen S**; Falgout Barry; Hanley Kathryn A; Blaney Jr Joseph E Jr; Markoff Lewis; Murphy Brian R. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.. swhitehead@niaid.nih.gov) . Journal of virology, (2003 Jan) Vol. 77, No. 2, pp. 1653-7. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB The Delta30 deletion mutation, which was originally created in dengue virus type 4 (DEN4) by the removal of nucleotides 172 to 143 from the 3' untranslated region (3' UTR), was introduced into a homologous region of wild-type (wt) dengue virus type 1 (DEN1). The resulting virus, rDEN1Delta30, was attenuated in rhesus monkeys to a level similar to that of the rDEN4Delta30 vaccine candidate. rDEN1Delta30 was more attenuated in rhesus monkeys than the previously described vaccine candidate, rDEN1mutF, which also contains mutations in the 3' UTR, and both vaccines were highly protective against challenge with wt DEN1. Both rDEN1Delta30 and rDEN1mutF were also attenuated in HuH-7-SCID mice. However, neither rDEN1Delta30 nor rDEN1mutF showed restricted replication following intrathoracic inoculation in the mosquito Toxorhynchites splendens. The ability of the Delta30 mutation to attenuate both DEN1 and DEN4 viruses suggests that a tetravalent DEN vaccine could be generated by introduction of the Delta30 mutation into wt DEN viruses belonging to each of the four serotypes.

L9 ANSWER 21 OF 22 MEDLINE on STN
2002445103. PubMed ID: 12202213. Genetic basis of attenuation of dengue virus type 4 small plaque mutants with restricted replication in suckling mice and in SCID mice transplanted with human liver cells. Blaney Joseph E Jr; Johnson Daniel H; Manipon Gracielle G; Firestone Cai-Yen; Hanson Christopher T; Murphy Brian R; **Whitehead Stephen S**. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-8007, USA.. jblaney@niaid.nih.gov) . Virology, (2002 Aug 15) Vol. 300, No. 1, pp. 125-39. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.
AB Mutations that restrict replication of dengue virus have been sought for the generation of recombinant live-attenuated dengue virus vaccines. Dengue virus type 4 (DEN4) was previously grown in Vero cells in the presence of 5-fluorouracil, and the characterization of 1248 mutagenized, Vero cell passaged clones identified 20 temperature-sensitive (ts) mutant viruses that were attenuated (att) in suckling mouse brain (J. E. Blaney, Jr., D. H. Johnson, C. Y. Firestone, C. T. Hanson, B. R. Murphy, and S. S. Whitehead, 2001, J. Virol. 75(20), 9731-9740). The present investigation has extended these studies by identifying an additional 22 DEN4 mutant viruses which have a small plaque size (sp) phenotype in Vero cells and/or the liver cell line, HuH-7. Five mutant viruses have a sp phenotype in both Vero and HuH-7 cells, three of which are also ts. Seventeen mutant viruses have a sp phenotype in only HuH-7 cells, 13 of which are also ts. Each of the sp viruses was growth restricted in the suckling mouse brain, exhibiting a wide range of reduction in replication (9- to 100,000-fold). Complete nucleotide sequence was determined for the 22 DEN4 sp mutant viruses, and nucleotide

all coding regions except NS4A. Identical mutations have been identified in multiple virus clones, suggesting that they may be involved in the adaptation of DEN4 virus to efficient growth in Vero cells. Six of the 22 sp 5-FU mutant viruses lacked coding mutations in the structural genes, and 17 recombinant DEN4 viruses were generated which separately encoded each of the mutations observed in these six sp viruses. Analysis of the recombinant DEN4 viruses defined the genetic basis of the sp, ts, and att phenotypes observed in the six sp viruses. Mutations in NS1, NS3, and the 3'-UTR were found to confer a greater than 100-fold, 10,000-fold, and 1000-fold reduction in replication of rDEN4 virus in SCID mice transplanted with HuH-7 cells, respectively, which serves as a novel small animal model for DEN4 infection.

L9 ANSWER 22 OF 22 MEDLINE on STN
2002003898. PubMed ID: 11752143. Paired charge-to-alanine mutagenesis of dengue virus type 4 NS5 generates mutants with temperature-sensitive, host range, and mouse attenuation phenotypes. Hanley Kathryn A; Lee Jay J; Blaney Joseph E Jr; Murphy Brian R; **Whitehead Stephen S**. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.. khanley@niaid.nih.gov) . Journal of virology, (2002 Jan) Vol. 76, No. 2, pp. 525-31. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Charge-to-alanine mutagenesis of dengue virus type 4 (DEN4) NS5 gene generated a collection of attenuating mutations for potential use in a recombinant live attenuated DEN vaccine. Codons for 80 contiguous pairs of charged amino acids in NS5 were individually mutagenized to create uncharged pairs of alanine residues, and 32 recombinant mutant viruses were recovered from the 80 full-length mutant DEN4 cDNA constructs. These mutant viruses were tested for temperature-sensitive (ts) replication in both Vero cells and HuH-7 human hepatoma cells. Of the 32 mutants, 13 were temperature sensitive (ts) in both cell lines, 11 were not ts in either cell line, and 8 exhibited a host range (tshr) phenotype. One tshr mutant was ts only in Vero cells, and seven were ts only in HuH-7 cells. Nineteen of the 32 mutants were 10-fold or more restricted in replication in the brains of suckling mice compared to that of wild-type DEN4, and three mutants were approximately 10,000-fold restricted in replication. The level of temperature sensitivity of replication in vitro did not correlate with attenuation in vivo. A virus bearing two pairs of charge-to-alanine mutations was constructed and demonstrated increased temperature sensitivity and attenuation relative to either parent virus. This large set of charge-to-alanine mutations specifying a wide range of attenuation for mouse brain should prove useful in fine-tuning recombinant live attenuated DEN vaccines.

```
=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     6.73      55.58

FILE 'USPATFULL' ENTERED AT 01:53:31 ON 10 JUL 2006
CA INDEXING COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 6 Jul 2006 (20060706/PD)
FILE LAST UPDATED: 6 Jul 2006 (20060706/ED)
HIGHEST GRANTED PATENT NUMBER: US7073200
HIGHEST APPLICATION PUBLICATION NUMBER: US2006150291
CA INDEXING IS CURRENT THROUGH 6 Jul 2006 (20060706/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 6 Jul 2006 (20060706/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2006
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2006


=> s dengue
L10        2809 DENGUE

=> s l10 and attenuat?
         193690 ATTENUAT?
L11         1267 L10 AND ATTENUAT?

=> s l11 and (temperature-sensitiv?)
        1597613 TEMPERATURE
         682535 SENSITIV?
          27311 TEMPERATURE-SENSITIV?
                (TEMPERATURE(W)SENSITIV?)
L12           95 L11 AND (TEMPERATURE-SENSITIV?)

=> s l12 and NS5
            599 NS5
L13          17 L12 AND NS5
```

L13 ANSWER 1 OF 17 USPATFULL on STN
2006:151575 Replication of hepatitis c virus in non-hepatic epithelial and mouse hepatic cells.
Zhu, Qing, Emeryville, CA, UNITED STATES
Guo, Ju-Tao, Lansdale, PA, UNITED STATES
Seeger, Christoph, Eikins Park, PA, UNITED STATES
US 2006128011 A1 20060615
APPLICATION: US 2003-536955 A1 20031212 (10)
WO 2003-US39722 20031212 20051018 PCT 371 date
PRIORITY: US 2002-433303P 20021213 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Cells and cell lines which replicate HCV of non-hepatic human and non human origin are disclosed. Also provided are methods of using such cells and cell lines to identify anti-HCV agents for the treatment of HCV infection.

L13 ANSWER 2 OF 17 USPATFULL on STN
2006:73763 Flavivirus variants having phenotypic variation and immunogenic compositions thereof.
Barrett, Alan D.T., Galveston, TX, UNITED STATES
Tesh, Robert B., Galveston, TX, UNITED STATES
Davis, C. Todd, Decatur, GA, UNITED STATES
Beasley, David W.C., Galveston, TX, UNITED STATES
Research Development Foundation, Carson City, NV, UNITED STATES (U.S. corporation)
US 2006062806 A1 20060323
APPLICATION: US 2005-223729 A1 20050909 (11)
PRIORITY: US 2004-608344P 20040909 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention concerns isolated **attenuated** flaviviruses, such as West Nile viruses, having modifications that provide phenotypic varation, particularly in comparison to a more virulent reference strains. The invention encompasses the isolated viruses and immunogenic compositions thereof, in addition to methods to produce and utilize same.

L13 ANSWER 3 OF 17 USPATFULL on STN
2006:73760 AVIRULENT, IMMUNOGENIC FLAVIVIRUS CHIMERAS.
Kinney, Richard M., Fort Collins, CO, UNITED STATES
Kinney, Claire Y.H., Fort Collins, CO, UNITED STATES
Gubler, Duane J., Fort Collins, CO, UNITED STATES
Butrapet, Siritorn, Bangkok, THAILAND
Bhamarapravati, Natth, Bangkok, THAILAND
US 2006062803 A1 20060323
APPLICATION: US 2001-204252 A1 20010216 (10)
WO 2001-US5142 20010216 20030129 PCT 371 date
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Chimeric flaviviruses that are avirulent and immunogenic are provided. The chimeric viruses are constructed to contain amino acid mutations in the nonstructural proteins of a flavivirus. Chimeric viruses containing the **attenuation**-mutated nonstructural genes of the virus are used as a backbone into which the structural protein genes of a second flavivirus strain are inserted. These chimeric viruses elicit pronounced immunogenicity yet lack the accompanying clinical symptoms of viral disease. The **attenuated** chimeric viruses are effective as immunogens or vaccines and may be combined in a pharmaceutical composition to confer simultaneous immunity against several strains of pathogenic flaviviruses.

L13 ANSWER 4 OF 17 USPATFULL on STN
2005:117619 Construction of West Nile virus and **dengue** virus chimeras for use in a live virus vaccine to prevent disease caused by West Nile virus.
Pletnev, Alexander G., Rockville, MD, UNITED STATES
Putnak, Joseph R., Silver Spring, MD, UNITED STATES
Chanock, Robert M., Bethesda, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
Blaney, Joseph E. JR., Frederick, MD, UNITED STATES
US 2005100886 A1 20050512
APPLICATION: US 2004-871775 A1 20040618 (10)
PRIORITY: US 2002-347281P 20020110 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to **attenuated**, immunogenic West Nile virus chimeras built on a **dengue** virus backbone for the production of immunogenic, live, **attenuated** West Nile virus vaccines.

L13 ANSWER 5 OF 17 USPATFULL on STN

Shi, Pei-Yong, Albany, NY, UNITED STATES
Lo, Michael, Albany, NY, UNITED STATES
Tilgner, Mark, Albany, NY, UNITED STATES
US 2005058987 A1 20050317
APPLICATION: US 2003-706892 A1 20031113 (10)
PRIORITY: US 2002-427117P 20021118 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The instant invention provides stable and novel lineage I WNV reverse genetics systems, and methods for making the reverse genetics systems, specifically, a fully-infectious lineage I WNV cDNA or replicon system engineered with one or more nucleotide sequences each encoding a reporter gene to be used in high throughput cell-based screening assays for the identification of novel antiflaviviral chemotherapeutics and/or vaccines effective to treat and/or immunize against infections by WNV and other emerging flaviviruses, such as, for example, JEV, SLEV, AV, KV, JV, CV, YV, TBEV, DENV-1, DENV-2, DENV-3, DENV-4,. YFV and MVEV. The present invention further provides methods of high throughput screening of antiflaviviral compounds or improved derivatives thereof using novel lineage I WNV reverse genetics systems and/or cell lines stably containing the reverse genetics systems. Also, the invention provides novel pharmaceutical compositions comprising an **attenuated** lineage I WNV that is less virulent but similarly immunogenic as the parent WNV and is capable of providing a protective immune response in a host.

L13 ANSWER 6 OF 17 USPATFULL on STN
2005:11907 Development of mutations useful for **attenuating dengue** viruses and chimeric **dengue** viruses.
Whitehead, Stephen S., Montgomery Village, MD, UNITED STATES
Murphy, Brian R., Bethesda, MD, UNITED STATES
Hanley, Kathryn A., Bethesda, MD, UNITED STATES
Blaney, Joseph E., Frederick, MD, UNITED STATES
US 2005010043 A1 20050113
APPLICATION: US 2003-719547 A1 20031121 (10)
PRIORITY: US 2001-293049P 20010522 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A menu of mutations was developed that is useful in fine-tuning the **attenuation** and growth characteristics of **dengue** virus vaccines.

L13 ANSWER 7 OF 17 USPATFULL on STN
2004:158167 Needleless vaccination using chimeric yellow fever vaccine-vectored vaccines against heterologous flaviviruses.
Mikszta, John A., Durham, NC, UNITED STATES
Alarcon, Jason B., Durham, NC, UNITED STATES
Dean, Cheryl, Raleigh, NC, UNITED STATES
Waterston, Andrea, Holly Springs, NC, UNITED STATES
Guirakhoo, Farshad, Melrose, ME, UNITED STATES
Thomas, Monath P., Harvard, MA, UNITED STATES
US 2004120964 A1 20040624
APPLICATION: US 2003-679036 A1 20031002 (10)
PRIORITY: US 2001-330713P 20011029 (60)
US 2001-333162P 20011127 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method of vaccinating a subject comprising delivering a chimeric yellow fever 17D strain vector expressing an envelope protein gene product of a heterologous flavivirus to the epidermal compartment or the intradermal compartment of the subject's skin. The invention encompasses vaccine compositions comprising the chimeric yellow fever viruses expressing an envelope protein gene product of a heterologous flavivirus. The compositions of the invention result in an enhanced therapeutic efficacy, e.g., enhanced protective immune response as they enhance the presentation and availability of the chimeric vaccine to the targeted compartment of the subject's skin.

L13 ANSWER 8 OF 17 USPATFULL on STN
2004:146867 Recombinant dimeric envelope vaccine against flaviviral infection.
Peters, Iain D., Bozeman, MT, United States
Coller, Beth-Ann G., Woluwe Saint Lambert, BELGIUM
McDonell, Michael, Bogart, GA, United States
Ivy, John M., College Station, TX, United States
Harada, Kent, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6749857 B1 20040615
APPLICATION: US 1999-376463 19990818 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention discloses and claims vaccines containing, as an active ingredient, a secreted recombinantly produced dimeric form of

eliciting the production of neutralizing antibodies against flaviviruses. The dimeric forms of truncated flaviviral envelope protein are formed 1) by directly linking two tandem copies of 80% E in a head to tail fashion via a flexible tether; 2) via the formation of a leucine zipper domain through the homodimeric association of two leucine zipper helices each fused to the carboxy terminus of an 80% E molecule; or 3) via the formation of a non-covalently associated four-helix bundle domain formed upon association of two helix-turn-helix moieties each attached to the carboxy terminus of an 80% E molecule. All products are expressed as a polyprotein including prM and the modified 80% E products are secreted from Drosophila melanogaster Schneider 2 cells using the human tissue plasminogen activator secretion signal sequence (tPA_L). Secreted products are generally more easily purified than those expressed intracellularly, facilitating vaccine production. One embodiment of the present invention is directed to a vaccine for protection of a subject against infection by **dengue** virus. The vaccine contains, as active ingredient, the dimeric form of truncated envelope protein of a **dengue** virus serotype. The dimeric truncated E is secreted as a recombinantly produced protein from eucaryotic cells. The vaccine may further contain portions of additional **dengue** virus serotype dimeric E proteins similarly produced. Another embodiment of the present invention is directed to methods to utilize the dimeric form of truncated **dengue** envelope protein for diagnosis of infection in individuals at risk for the disease. The diagnostic contains, as active ingredient, the dimeric form of truncated envelope protein of a **dengue** virus serotype. The dimeric truncated E is secreted as a recombinantly produced protein from eucaryotic cells. The diagnostic may further contain portions of additional **dengue** virus serotype dimeric E proteins similarly produced.

L13 ANSWER 9 OF 17 USPATFULL on STN
2004:9469 Chimeric and/or growth-restricted flaviviruses.
Lai, Ching-Juh, Bethesda, MD, United States
Bray, Michael, Bethesda, MD, United States
Pletnev, Alexander G., Rockville, MD, United States
Men, Ruhe, Rockville, MD, United States
Zhang, Yi-Ming, Falls Church, VA, United States
Eckels, Kenneth H., Rockville, MD, United States
Chanock, Robert M., Bethesda, MD, United States
The United States of America as represented by the Department of Health and Human Services., Washington, DC, United States (U.S. government)
US 6676936 B1 20040113
APPLICATION: US 2000-643217 20000818 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention includes a chimeric virus for use in a vaccine preparation having a genome comprising nucleic acid sequences encoding at least one structural protein from one flavivirus and nucleic acid sequences encoding nonstructural protein from another flavivirus. The genome preferably includes mutations within the viral genome that reduce virus virulence and in a particularly preferred embodiment these vaccines are directed to flaviviruses such as **dengue** virus, tick-borne encephalitis virus and Japanese encephalitis virus. The invention also includes a baculovirus having a recombinant **dengue** cDNA sequence which encodes: (1) **dengue** virus capsid protein, pre-matrix protein, envelope glycoprotein and NS1 and NS2a nonstructural proteins or (2) **dengue** envelope glycoprotein or (3) **dengue** non-structural proteins NS1 and NS2a. The invention further includes a baculovirus having a recombinant Japanese B encephalitis virus cDNA sequence which encodes the Japanese B encephalitis virus capsid protein, pre-matrix protein, envelope glycoprotein and non-structural proteins NS1 and NS2a. The invention further includes a vaccine and a method to produce that vaccine.

L13 ANSWER 10 OF 17 USPATFULL on STN
2003:276776 Use of flavivirus for the expression of protein epitopes and development of new live **attenuated** vaccine virus to immune against flavivirus and other infectious agents.
Bonaldo, Mirna C., Rio de Janeiro, BRAZIL
Galler, Ricardo, Rio de Janeiro, BRAZIL
Freire, Marcos da Silva, Rio de Janeiro, BRAZIL
Garrat, Richard C., Sao Paulo, BRAZIL
US 2003194801 A1 20031016
APPLICATION: US 2003-275707 A1 20030410 (10)
WO 2002-BR36 20020308
PRIORITY: GB 2001-5877 20010309
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a vaccine against infections caused by flavivirus. More particularly to the use of the YF vaccine virus (17D) to express at the level of its envelope, protein epitopes from other pathogens which will elicit a specific immune response to the parental pathogen.

L13 ANSWER 11 OF 17 USPATFULL on STN
2003:250518 Recombinant dimeric envelope vaccine against flaviviral infection.
Peters, Iain D., Bozeman, MT, UNITED STATES
Coller, Beth-Ann G., Woluwe Saint Lambert, BELGIUM
McDonell, Michael, Bogart, GA, UNITED STATES
Ivy, John M., College Station, TX, UNITED STATES
Harada, Kent, Honolulu, HI, UNITED STATES
US 2003175304 A1 20030918
APPLICATION: US 2002-247960 A1 20020920 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention discloses and claims vaccines containing, as an active ingredient, a secreted recombinantly produced dimeric form of truncated flaviviral envelope protein. The vaccines are capable of eliciting the production of neutralizing antibodies against flaviviruses. The dimeric forms of truncated flaviviral envelope protein are formed 1) by directly linking two tandem copies of 80% E in a head to tail fashion via a flexible tether; 2) via the formation of a leucine zipper domain through the homodimeric association of two leucine zipper helices each fused to the carboxy terminus of an 80% E molecule; or 3) via the formation of a non-covalently associated four-helix bundle domain formed upon association of two helix-turn-helix moieties each attached to the carboxy terminus of an 80% E molecule. All products are expressed as a polyprotein including prM and the modified 80% E products are secreted from Drosophila melanogaster Schneider 2 cells using the human tissue plasminogen activator secretion signal sequence (tPA_L). Secreted products are generally more easily purified than those expressed intracellularly, facilitating vaccine production. One embodiment of the present invention is directed to a vaccine for protection of a subject against infection by **dengue** virus. The vaccine contains, as active ingredient, the dimeric form of truncated envelope protein of a **dengue** virus serotype. The dimeric truncated E is secreted as a recombinantly produced protein from eucaryotic cells. The vaccine may further contain portions of additional **dengue** virus serotype dimeric E proteins similarly produced. Another embodiment of the present invention is directed to methods to utilize the dimeric form of truncated **dengue** envelope protein for diagnosis of infection in individuals at risk for the disease. The diagnostic contains, as active ingredient, the dimeric form of truncated envelope protein of a **dengue** virus serotype. The dimeric truncated E is secreted as a recombinantly produced protein from eucaryotic cells. The diagnostic may further contain portions of additional **dengue** virus serotype dimeric E proteins similarly produced.

L13 ANSWER 12 OF 17 USPATFULL on STN
2003:183854 Vaccines against infections caused by YF virus; YF infectious cDNA, method for producing a recombinant YF virus from the YF infectious cDNA and plasmids to assemble the YF infectious cDNA.
Galler, Ricardo, Niteroi, BRAZIL
Freire, Marcos Da Silva, Niteroi, BRAZIL
Fundacao Oswaldo Cruz-Fiocruz, Rio de Janeiro, BRAZIL (non-U.S. corporation)
US 6589522 B1 20030708
APPLICATION: US 2000-705949 20001106 (9)
PRIORITY: BR 1997-1774 19970411
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is related to a vaccine composition for humans against YF infections consisting essencially of a recombinant YF virus, YFiv5.2/DD, which is regenerated from YF infectious cDNA. There is provided new plasmids, pYF 5'3' IV/G1/2 and pYFM 5.2/T3/27, which together, have the complete sequence of said YF infectious cDNA. The method for producing recombinant YF virus and the Original, Primary and Secondary Seed Lots are other embodiments of the present invention.

L13 ANSWER 13 OF 17 USPATFULL on STN
2001:18271 Chimeric and/or growth-restricted flaviviruses.
Lai, Ching-Juh, Bethesda, MD, United States
Bray, Michael, Bethesda, MD, United States
Pletnev, Alexander G., Rockville, MD, United States
Men, Ruhe, Kensington, MD, United States
Zhang, Yi-Ming, Bethesda, MD, United States
Eckels, Kenneth H., Bethesda, MD, United States
Chanock, Robert M., Bethesda, MD, United States
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6184024 B1 20010206
APPLICATION: US 1994-250802 19940527 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention includes a chimeric virus for use in a vaccine preparation having a genome comprising nucleic acid sequences encoding at least one

encoding nonstructural protein from another flavivirus. The genome preferably includes mutations within the viral genome that reduce virus virulence and in a particularly preferred embodiment these vaccines are directed to flaviviruses such as **dengue** virus, tick-borne encephalitis virus and Japanese encephalitis virus. The invention also includes a baculovirus having a recombinant **dengue** cDNA sequence which encodes: (1) **dengue** virus capsid protein, pre-matrix protein, envelope glycoprotein and NS1 and NS2a nonstructural proteins or (2) **dengue** envelope glycoprotein or (3) **dengue** non-structural proteins NS1 and NS2a. The invention further includes a baculovirus having a recombinant Japanese B encephalitis virus cDNA sequence which encodes the Japanese B encephalitis virus capsid protein, pre-matrix protein, envelope glycoprotein and non-structural proteins NS1 and NS2a. The invention further includes a vaccine and a method to produce that vaccine.

L13 ANSWER 14 OF 17 USPATFULL on STN
2001:4528 Yellow fever infectious cDNA and plasmids.
Galler, Ricardo, Niteroi, Brazil
Freire, Marcos Da Silva, Niteroi, Brazil
Fundaco Oswaldo Cruz-Fiocruz, Rio de Janiero, Brazil (non-U.S. corporation)
US 6171854 B1 20010109
APPLICATION: US 1998-58411 19980410 (9)
PRIORITY: BR 1997-1774 19970411
DOCUMENT TYPE: Patent; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is related to a vaccine composition for humans against YF infections consisting essentially of a recombinant YF virus, YFiv5.2/DD, which is regenerated from YF infectious cDNA. There are provided new plasmids, pYF 5'3' IV/G1/2 and pYFM 5.2/T3/27, which together, have the complete sequence of said YF infectious cDNA. The method for producing recombinant YF virus and the Original, Primary and Secondary Seed Lots are other embodiments of the present invention.

L13 ANSWER 15 OF 17 USPATFULL on STN
2000:174106 Subunit immonogenic composition against **dengue** infection.
Ivy, John, Kailua, HI, United States
Nakano, Eilen, Hon., HI, United States
Clements, David, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6165477 20001226
APPLICATION: US 1997-915152 19970820 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The Flaviviridae comprise a number of medically important pathogens that cause significant morbidity in humans including the **dengue** (DEN) virus, Japanese encephalitis (JE) virus, tick-borne encephalitis virus (TBE), and yellow fever virus (YF). Flaviviruses are generally transmitted to vertebrates by chronically infected mosquito or tick vectors. The viral particle which is enveloped by host cell membranes, comprises a single positive strand genomic RNA and the structural capsid (CA), membrane (M), and envelope (E) proteins. The E and M proteins are found on the surface of the virion where they are anchored in the membrane. Mature E is glycosylated and contains functional domains responsible for cell surface attachment and intraendosomal fusion activities. Problems have arisen in the art with respect to producing recombinant forms of the E glycoprotein that retain their native configuration and attendant properties associated therewith (i.e., ability to induce neutralizing antibody responses). To date, recombinantly produced E glycoproteins have suffered from a number of limitations including improper glycosylation, folding, and disulfide bond formation. The claimed invention has addressed these concerns by providing secreted recombinant forms of the E glycoprotein that are highly immunogenic and appear to retain their native configuration. Carboxy-terminally truncated forms of E containing the amino terminal 395 amino acids and a suitable secretion signal sequence were generated in Drosophila melanogaster Schneider cell lines. Immunogenic compositions comprising these recombinant envelope glycoproteins were capable of inducing protective, neutralizing antibody responses when administered to a suitable host.

L13 ANSWER 16 OF 17 USPATFULL on STN
2000:142128 Methods of preparing carboxy-terminally truncated recombinant flavivirus envelope glycoproteins employing drosophila melanogaster expression systems.
Ivy, John, Kailua, HI, United States
Nakano, Eilen, Honolulu, HI, United States
Clements, David, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6136561 20001024
APPLICATION: US 1997-937195 19970925 (8)

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The Flaviviridae comprise a number of medically important pathogens that cause significant morbidity in humans including the **dengue** (DEN) virus, Japanese encephalitis (JE) virus, tick-borne encephalitis virus (TBE), and yellow fever virus (YF). Flaviviruses are generally transmitted to vertebrates by chronically infected mosquito or tick vectors. The viral particle which is enveloped by host cell membranes, comprises a single positive strand genomic RNA and the structural capsid (CA), membrane (M), and envelope (E) proteins. The E and M proteins are found on the surface of the virion where they are anchored in the membrane. Mature E is glycosylated and contains functional domains responsible for cell surface attachment and intraendosomal fusion activities. Problems have arisen in the art with respect to producing recombinant forms of the E glycoprotein that retain their native configuration and attendant properties associated therewith (i.e., ability to induce neutralizing antibody responses). To date, recombinantly produced E glycoproteins have suffered from a number of limitations including improper glycosylation, folding, and disulfide bond formation. The claimed invention has addressed these concerns by providing secreted recombinant forms of the E glycoprotein that are highly immunogenic and appear to retain their native configuration. Carboxy-terminally truncated forms of E containing the amino terminal 395 amino acids and a suitable secretion signal sequence were generated in Drosophila melanogaster Schneider cell lines. The recombinant proteins produced by this expression system should prove useful, inter alia, as immunogens and diagnostic reagents.

L13 ANSWER 17 OF 17 USPATFULL on STN
2000:121283 Recombinant vaccine made in E. coli against **dengue** virus.
Srivastava, Ashok Kumar, Silver Spring, MD, United States
Putnak, J. Robert, Silver Spring, MD, United States
Hoke, Charles H., Columbia, MD, United States
Warren, Richard L., Brookville, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. corporation)
US 6117640 20000912
APPLICATION: US 1995-433263 19950502 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A recombinant protein encompassing a C-terminal protion from the structural envelope glycoprotein and an N-terminal portion from non-structural protein one of **dengue** type 2 virus was expressed in Escherichia coli as a fusion protein with Staphylococcal protein A. The recombinant protein was found to provide protection against lethal challenge with **dengue** 2 in mice.

=> d his

(FILE 'HOME' ENTERED AT 01:43:33 ON 10 JUL 2006)

FILE 'USPATFULL' ENTERED AT 01:43:53 ON 10 JUL 2006
E WHITEHEAD STEPHEN S/IN
L1 14 S E3
E MURPHY BRIAN R/IN
L2 32 S E3
L3 3 S L2 AND DENGUE
E HANLEY KATHRYN A/IN
L4 1 S E3
E BLANEY JOSEPH E/IN
L5 2 S E3-E4
L6 0 S L5 NOT (L1 OR L2)

FILE 'WPIDS' ENTERED AT 01:48:19 ON 10 JUL 2006
E WHITEHEAD S S/IN
L7 9 S E3
L8 9 S L7 NOT (L1 OR L2)

FILE 'MEDLINE' ENTERED AT 01:50:04 ON 10 JUL 2006
E WHITEHEAD S S/AU
E WHITEHEAD STEPHEN/AU
L9 22 S E4-E5

FILE 'USPATFULL' ENTERED AT 01:53:31 ON 10 JUL 2006
L10 2809 S DENGUE
L11 1267 S L10 AND ATTENUAT?
L12 95 S L11 AND (TEMPERATURE-SENSITIV?)
L13 17 S L12 AND NS5

=> file medline
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION

```
FILE 'MEDLINE' ENTERED AT 01:56:04 ON 10 JUL 2006

 FILE LAST UPDATED: 8 JUL 2006 (20060708/UP).  FILE COVERS 1950 TO DATE.

 On December 11, 2005, the 2006 MeSH terms were loaded.

 The MEDLINE reload for 2006 is now (26 Feb.) available.  For details
 on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
 See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

 OLDMEDLINE is covered back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2006 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> file medline
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                 0.39      95.69

FILE 'MEDLINE' ENTERED AT 01:56:41 ON 10 JUL 2006

 FILE LAST UPDATED: 8 JUL 2006 (20060708/UP).  FILE COVERS 1950 TO DATE.

 On December 11, 2005, the 2006 MeSH terms were loaded.

 The MEDLINE reload for 2006 is now (26 Feb.) available.  For details
 on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
 See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

 OLDMEDLINE is covered back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2006 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> s dengue
L14        5188 DENGUE

=> s l14 and attenuat?
         118537 ATTENUAT?
L15         196 L14 AND ATTENUAT?

=> d his

     (FILE 'HOME' ENTERED AT 01:43:33 ON 10 JUL 2006)

     FILE 'USPATFULL' ENTERED AT 01:43:53 ON 10 JUL 2006
                E WHITEHEAD STEPHEN S/IN
L1             14 S E3
                E MURPHY BRIAN R/IN
L2             32 S E3
L3              3 S L2 AND DENGUE
                E HANLEY KATHRYN A/IN
L4              1 S E3
                E BLANEY JOSEPH E/IN
L5              2 S E3-E4
L6              0 S L5 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 01:48:19 ON 10 JUL 2006
                E WHITEHEAD S S/IN
L7              9 S E3
L8              9 S L7 NOT (L1 OR L2)

     FILE 'MEDLINE' ENTERED AT 01:50:04 ON 10 JUL 2006
                E WHITEHEAD S S/AU
```

```
L9          22 S E4-E5

     FILE 'USPATFULL' ENTERED AT 01:53:31 ON 10 JUL 2006
L10       2809 S DENGUE
L11       1267 S L10 AND ATTENUAT?
L12         95 S L11 AND (TEMPERATURE-SENSITIV?)
L13         17 S L12 AND NS5

     FILE 'MEDLINE' ENTERED AT 01:56:04 ON 10 JUL 2006

     FILE 'MEDLINE' ENTERED AT 01:56:41 ON 10 JUL 2006
L14       5188 S DENGUE
L15        196 S L14 AND ATTENUAT?

=> s l15 and (temperature-sensitiv?)
        292979 TEMPERATURE
        713571 SENSITIV?
         13176 TEMPERATURE-SENSITIV?
                 (TEMPERATURE(W)SENSITIV?)
L16        22 L15 AND (TEMPERATURE-SENSITIV?)

=> s l16 not l13
          5188 DENGUE
        118537 ATTENUAT?
        292979 TEMPERATURE
        713571 SENSITIV?
         13176 TEMPERATURE-SENSITIV?
                 (TEMPERATURE(W)SENSITIV?)
           545 NS5
L17        20 L16 NOT L13

=> d l17,cbib,ab,1-20

L17  ANSWER 1 OF 20     MEDLINE on STN
2004040700.     PubMed ID: 14740949.   Biologic properties of dengue viruses
     following serial passage in primary dog kidney cells: studies at the
     University of Hawaii. Halstead Scott B; Marchette Nyven J. (Department of
     Tropical Medicine and Medical Microbiology, John A. Burns School of
     Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA..
     halsteads@erols.com) . The American journal of tropical medicine and
     hygiene, (2003 Dec) Vol. 69, No. 6 Suppl, pp. 5-11.  Journal code:
     0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB   Serial passage at low dilution of seven different wild-type dengue (DEN)
     viruses into primary dog kidney (PDK) cell cultures placed selective
     pressure that resulted in the following changes from parental phenotype:
     smaller plaques in LLC-MK2 cells, absent plaque formation in green monkey
     kidney cells, lack of a cytopathic effect in LLC-MK2 cells, shut-off of
     virus replication at high temperatures (temperature sensitivity),
     reduced virulence for rhesus monkeys manifested by reduced or absent
     viremia and/or absence of a secondary-type antibody response following
     homotypic challenge, and progressive increase in the mean day of death
     following intracerebral inoculation of sucking mice.  Two DEN-1 strains
     showed most of these changes by the 15th PDK passage.  Only one of two
     DEN-2 strains studied was carried to the 50th PDK passage at the
     University of Hawaii.  For the latter strain, both the temperature of
     viral replicative shutoff and mouse neurovirulence were reduced.  Three
     DEN-4 strains showed similar late-passage biologic marker changes.  The
     observations made, although not exhaustive, provide laboratory correlates
     for virus strains that have shown reduced virulence but retained
     immunogenicity in humans.  Candidate human vaccines have been prepared
     from five of the studied strains: DEN-1 (16007) at PDK 13, DEN-2 (16681
     and S-16803) at PDK 50 or above, and DEN-4 (1036 and 341750) at PDK 48 and
     20, respectively.

L17  ANSWER 2 OF 20     MEDLINE on STN
2003510478.     PubMed ID: 14557629.   Dengue 2 PDK-53 virus as a chimeric
     carrier for tetravalent dengue vaccine development. Huang Claire Y-H;
     Butrapet Siritorn; Tsuchiya Kiyotaka R; Bhamarapravati Natth; Gubler Duane
     J; Kinney Richard M. (Division of Vector-Borne Infectious Diseases,
     Centers for Disease Control and Prevention, Public Health Service, U.S.
     Department of Health and Human Services, Fort Collins, Colorado 80522,
     USA.. chuangl@cdc.gov) . Journal of virology, (2003 Nov) Vol. 77, No. 21,
     pp. 11436-47.  Journal code: 0113724. ISSN: 0022-538X. Pub. country:
     United States. Language: English.
AB   Attenuation markers of the candidate dengue 2 (D2) PDK-53 vaccine
     virus are encoded by mutations that reside outside of the structural gene
     region of the genome.  We engineered nine dengue virus chimeras
     containing the premembrane (prM) and envelope (E) genes of wild-type D1
     16007, D3 16562, or D4 1036 virus within the genetic backgrounds of
     wild-type D2 16681 virus and the two genetic variants (PDK53-E and
     PDK53-V) of the D2 PDK-53 vaccine virus.  Expression of the heterologous
     prM-E genes in the genetic backgrounds of the two D2 PDK-53 variants, but
```

retained PDK-53 characteristic phenotypic markers of **attenuation**, including small plaque size and **temperature sensitivity** in LLC-MK(2) cells, limited replication in C6/36 cells, and lack of neurovirulence in newborn ICR mice. Chimeric D2/1, D2/3, and D2/4 viruses replicated efficiently in Vero cells and were immunogenic in AG129 mice. Chimeric D2/1 viruses protected adult AG129 mice against lethal D1 virus challenge. Two tetravalent virus formulations, comprised of either PDK53-E- or PDK53-V-vectored viruses, elicited neutralizing antibody titers in mice against all four **dengue** serotypes. These antibody titers were similar to the titers elicited by monovalent immunizations, suggesting that viral interference did not occur in recipients of the tetravalent formulations. The results of this study demonstrate that the unique **attenuation** loci of D2 PDK-53 virus make it an attractive vector for the development of live **attenuated** flavivirus vaccines.

L17 ANSWER 3 OF 20 MEDLINE on STN

2002445103. PubMed ID: 12202213. Genetic basis of **attenuation** of **dengue** virus type 4 small plaque mutants with restricted replication in suckling mice and in SCID mice transplanted with human liver cells. Blaney Joseph E Jr; Johnson Daniel H; Manipon Gracielle G; Firestone Cai-Yen; Hanson Christopher T; Murphy Brian R; Whitehead Stephen S. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-8007, USA.. jblaney@niaid.nih.gov) . Virology, (2002 Aug 15) Vol. 300, No. 1, pp. 125-39. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Mutations that restrict replication of **dengue** virus have been sought for the generation of recombinant live-**attenuated dengue** virus vaccines. **Dengue** virus type 4 (DEN4) was previously grown in Vero cells in the presence of 5-fluorouracil, and the characterization of 1248 mutagenized, Vero cell passaged clones identified 20 **temperature-sensitive** (ts) mutant viruses that were **attenuated** (att) in suckling mouse brain (J. E. Blaney, Jr., D. H. Johnson, C. Y. Firestone, C. T. Hanson, B. R. Murphy, and S. S. Whitehead, 2001, J. Virol. 75(20), 9731-9740). The present investigation has extended these studies by identifying an additional 22 DEN4 mutant viruses which have a small plaque size (sp) phenotype in Vero cells and/or the liver cell line, HuH-7. Five mutant viruses have a sp phenotype in both Vero and HuH-7 cells, three of which are also ts. Seventeen mutant viruses have a sp phenotype in only HuH-7 cells, 13 of which are also ts. Each of the sp viruses was growth restricted in the suckling mouse brain, exhibiting a wide range of reduction in replication (9- to 100,000-fold). Complete nucleotide sequence was determined for the 22 DEN4 sp mutant viruses, and nucleotide substitutions were found in the 3'-untranslated region (UTR) as well as in all coding regions except NS4A. Identical mutations have been identified in multiple virus clones, suggesting that they may be involved in the adaptation of DEN4 virus to efficient growth in Vero cells. Six of the 22 sp 5-FU mutant viruses lacked coding mutations in the structural genes, and 17 recombinant DEN4 viruses were generated which separately encoded each of the mutations observed in these six sp viruses. Analysis of the recombinant DEN4 viruses defined the genetic basis of the sp, ts, and att phenotypes observed in the six sp viruses. Mutations in NS1, NS3, and the 3'-UTR were found to confer a greater than 100-fold, 10,000-fold, and 1000-fold reduction in replication of rDEN4 virus in SCID mice transplanted with HuH-7 cells, respectively, which serves as a novel small animal model for DEN4 infection.

L17 ANSWER 4 OF 20 MEDLINE on STN

2001512173. PubMed ID: 11559806. Chemical mutagenesis of **dengue** virus type 4 yields mutant viruses which are **temperature sensitive** in vero cells or human liver cells and **attenuated** in mice. Blaney J E Jr; Johnson D H; Firestone C Y; Hanson C T; Murphy B R; Whitehead S S. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.. jblaney@niaid.nih.gov) . Journal of virology, (2001 Oct) Vol. 75, No. 20, pp. 9731-40. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB A recombinant live **attenuated dengue** virus type 4 (DEN4) vaccine candidate, 2ADelta30, was found previously to be generally well tolerated in humans, but a rash and an elevation of liver enzymes in the serum occurred in some vaccinees. 2ADelta30, a non-**temperature-sensitive** (non-ts) virus, contains a 30-nucleotide deletion (Delta30) in the 3' untranslated region (UTR) of the viral genome. In the present study, chemical mutagenesis of DEN4 was utilized to generate **attenuating** mutations which may be useful in further **attenuation** of the 2ADelta30 candidate vaccine. Wild-type DEN4 2A virus was grown in Vero cells in the presence of 5-fluorouracil, and a panel of 1,248 clones were isolated. Twenty ts mutant viruses were identified that were ts in both simian Vero and human liver HuH-7 cells (n = 13) or only in HuH-7 cells (n = 7). Each of the 20 ts mutant viruses possessed an **attenuation** phenotype, as indicated by restricted replication in the brains of 7-day-old mice. The complete nucleotide sequence of the 20 ts mutant viruses identified

in the 5' and 3' UTRs, with more than one change occurring, in general, per mutant virus. A ts mutation in the NS3 protein (nucleotide position 4995) was introduced into a recombinant DEN4 virus possessing the Delta30 deletion, thereby creating rDEN4Delta30-4995, a recombinant virus which is ts and more **attenuated** than rDEN4Delta30 virus in the brains of mice. We are assembling a menu of **attenuating** mutations that should be useful in generating satisfactorily **attenuated** recombinant **dengue** vaccine viruses and in increasing our understanding of the pathogenesis of **dengue** virus.

L17 ANSWER 5 OF 20 MEDLINE on STN
2001253021. PubMed ID: 11285157. Study of biologic attributes of Cuban **dengue** 2 virus after serial passage in primary dog kidney cells. Alvarez M; Guzman M G; Pupo M; Morier L; Bravo J; Rodriguez R. (Department of Virology, PAHO/WHO Collaborator Center for Viral Diseases, Tropical Medicine Institute of Havana, Cuba.) International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases, (2001) Vol. 5, No. 1, pp. 35-9. Journal code: 9610933. ISSN: 1201-9712. Pub. country: Canada. Language: English.
AB OBJECTIVE: The serial passage of **dengue** viruses in primary dog kidney (PDK) cells has resulted in selection of **attenuated** viruses. However, the molecular changes responsible for loss of virulence are not well characterized. This article describes the isolation and biologic attributes of one **dengue** 2 virulent strain as a first step to allow the study of determinants of virulence at the molecular level. METHODS: A15 **dengue** 2 Cuban strain was isolated from the viremic plasma of a patient with uncomplicated **dengue** fever during the 1981 epidemic. This was then subjected to serial passage in PDK cells. Viruses resulting from several PDK passages were compared to the parent strain for plaque size and **temperature sensitivity**, neurovirulence in newborn mice, and cytopathogenic effects on LLC-MK(2) and C6/36-HT cell lines. RESULTS: A15 **dengue** 2 Cuban strain was successfully propagated in PDK cells. Primary dog kidney 52 to 53 viruses exhibited several biologic attributes, such as small plaques, **temperature sensitivity**, reduced mouse neurovirulence, and cytopathic effect in permissive cell lines. CONCLUSIONS: These results represent the first step to allow **attenuation** of this strain of **dengue** 2 virus.

L17 ANSWER 6 OF 20 MEDLINE on STN
2000173690. PubMed ID: 10708416. Chimeric **dengue** type 2 (vaccine strain PDK-53)/**dengue** type 1 virus as a potential candidate **dengue** type 1 virus vaccine. Huang C Y; Butrapet S; Pierro D J; Chang G J; Hunt A R; Bhamarapravati N; Gubler D J; Kinney R M. (Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Fort Collins, Colorado 80522, USA.) Journal of virology, (2000 Apr) Vol. 74, No. 7, pp. 3020-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB We constructed chimeric **dengue** type 2/type 1 (DEN-2/DEN-1) viruses containing the nonstructural genes of DEN-2 16681 virus or its vaccine derivative, strain PDK-53, and the structural genes (encoding capsid protein, premembrane protein, and envelope glycoprotein) of DEN-1 16007 virus or its vaccine derivative, strain PDK-13. We previously reported that **attenuation** markers of DEN-2 PDK-53 virus were encoded by genetic loci located outside the structural gene region of the PDK-53 virus genome. Chimeric viruses containing the nonstructural genes of DEN-2 PDK-53 virus and the structural genes of the parental DEN-1 16007 virus retained the **attenuation** markers of small plaque size and **temperature sensitivity** in LLC-MK(2) cells, less efficient replication in C6/36 cells, and **attenuation** for mice. These chimeric viruses elicited higher mouse neutralizing antibody titers against DEN-1 virus than did the candidate DEN-1 PDK-13 vaccine virus or chimeric DEN-2/DEN-1 viruses containing the structural genes of the PDK-13 virus. Mutations in the envelope protein of DEN-1 PDK-13 virus affected in vitro phenotype and immunogenicity in mice. The current PDK-13 vaccine is the least efficient of the four Mahidol candidate DEN virus vaccines in human trials. The chimeric DEN-2/DEN-1 virus might be a potential DEN-1 virus vaccine candidate. This study indicated that the infectious clones derived from the candidate DEN-2 PDK-53 vaccine are promising **attenuated** vectors for development of chimeric flavivirus vaccines.

L17 ANSWER 7 OF 20 MEDLINE on STN
2000173689. PubMed ID: 10708415. **Attenuation** markers of a candidate **dengue** type 2 vaccine virus, strain 16681 (PDK-53), are defined by mutations in the 5' noncoding region and nonstructural proteins 1 and 3. Butrapet S; Huang C Y; Pierro D J; Bhamarapravati N; Gubler D J; Kinney R M. (Center for Vaccine Development, Institute of Science and Technology for Development, Mahidol University at Salaya, Nakhonpathom 73170, Thailand.) Journal of virology, (2000 Apr) Vol. 74, No. 7, pp. 3011-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB The genome of a candidate **dengue** type 2 (DEN-2) vaccine virus, strain

infectious cDNA clones, we constructed 18 recombinant 16681/PDK-53 viruses to analyze four 16681-to-PDK-53 mutations, including 5' noncoding region (5'NC)-57 C-to-T, premembrane (prM)-29 Asp-to-Val (the only mutation that occurs in the structural proteins), nonstructural protein 1 (NS1)-53 Gly-to-Asp, and NS3-250 Glu-to-Val. The viruses were studied for plaque size, growth rate, and **temperature sensitivity** in LLC-MK(2) cells, growth rate in C6/36 cells, and neurovirulence in newborn mice. All of the viruses replicated to peak titers of 10(7.3) PFU/ml or greater in LLC-MK(2) cells. The crippled replication of PDK-53 virus in C6/36 cells and its **attenuation** for mice were determined primarily by the 5'NC-57-T and NS1-53-Asp mutations. The **temperature sensitivity** of PDK-53 virus was attributed to the NS1-53-Asp and NS3-250-Val mutations. The 5'NC-57, NS1-53, and NS3-250 loci all contributed to the small-plaque phenotype of PDK-53 virus. Reversions at two or three of these loci in PDK-53 virus were required to reconstitute the phenotypic characteristics of the parental 16681 virus. The prM-29 locus had little or no effect on viral phenotype. Sequence analyses showed that PDK-53 virus is genetically identical to PDK-45 virus. Restriction of the three major genetic determinants of **attenuation** markers to nonstructural genomic regions makes the PDK-53 virus genotype attractive for the development of chimeric DEN virus vaccine candidates.

L17 ANSWER 8 OF 20 MEDLINE on STN

97288308. PubMed ID: 9143286. Construction of infectious cDNA clones for **dengue** 2 virus: strain 16681 and its **attenuated** vaccine derivative, strain PDK-53. Kinney R M; Butrapet S; Chang G J; Tsuchiya K R; Roehrig J T; Bhamarapravati N; Gubler D J. (Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado, USA.. rmk1@cdc.gov) . Virology, (1997 Apr 14) Vol. 230, No. 2, pp. 300-8. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB We identified nine nucleotide differences between the genomes of **dengue**-2 (DEN-2) 16681 virus and its vaccine derivative, strain PDK-53. These included a C-to-T (16681-to-PDK-53) mutation at nucleotide position 57 of the 5'-untranslated region, three silent mutations, and substitutions prM-29 Asp to Val, NS1-53 Gly to Asp, NS2A-181 Leu to Phe, NS3-250 Glu to Val, and NS4A-75 Gly to Ala. Unpassaged PDK-53 vaccine contained two genetic variants as a result of partial mutation at NS3-250. We constructed infectious cDNA clones for 16681 virus and each of the two PDK-53 variants. DEN-2 16681 clone-derived viruses were identical to the 16681 virus in plaque size and replication in LLC-MK2 cells, replication in C6/36 cells, E and prM epitopes, and neurovirulence for suckling mice. PDK-53 virus and both clone-derived PDK-53 variants were **attenuated** in mice. However, the variant containing NS3-250-Glu was less **temperature sensitive** and replicated better in C6/36 cells than did PDK-53 virus. The variant containing NS3-250-Val had smaller, more diffuse plaques, decreased replication, and increased **temperature sensitivity** in LLC-MK2 cells relative to PDK-53 virus. Both PDK-53 virus and the NS3-250-Val variant replicated poorly in C6/36 cells relative to 16681 virus. Unpassaged PDK-53 vaccine virus and the virus passaged once in LLC-MK2 cells had genomes of identical sequence, including the mixed NS3-250-Glu/Val locus. Although the NS3-250-Val mutation clearly affected virus replication in vitro, it was not a major determinant of **attenuation** for PDK-53 virus in suckling mice.

L17 ANSWER 9 OF 20 MEDLINE on STN

91345181. PubMed ID: 1877715. Infection of Aedes albopictus and Aedes aegypti mosquitoes with **dengue** parent and progeny candidate vaccine viruses: a possible marker of human **attenuation**. Schoepp R J; Beaty B J; Eckels K H. (Department of Microbiology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins.) The American journal of tropical medicine and hygiene, (1991 Aug) Vol. 45, No. 2, pp. 202-10. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB **Dengue** (DEN-1) and DEN-4 parent (P) and progeny candidate vaccine (CV) viruses were compared in their abilities to infect and to replicate in Aedes aegypti and Aedes albopictus mosquitoes. The DEN CV clones were **temperature sensitive** (ts) and had small plaque morphology. The DEN-1 and DEN-4 CV viruses differed in their ability to infect, to replicate in, and to be transmitted by mosquitoes. The DEN-1 CV virus was not **attenuated** for the vector mosquitoes; oral infection rates with the CV virus were as high as or higher than the P virus, and the CV virus replicated efficiently in mosquitoes after oral infection. The DEN-4 CV virus was **attenuated**; it was less efficient than its P virus in infection and replication in mosquitoes. Thus, the ts phenotype and small plaque morphology are not reliable biological markers for prediction of vector **attenuation**. Similar results were reported by others for **attenuation** in man and monkeys. These studies with DEN-1 and DEN-4 viruses, and previously reported studies with DEN-2 virus and with DEN-3 virus suggest that vector and vertebrate host **attenuation** are genetically linked. Thus, vector **attenuation** may be a biological marker for human **attenuation**.

L17 ANSWER 10 OF 20 MEDLINE on STN
90145351. PubMed ID: 2301711. **Dengue** 3 virus infection of Aedes albopictus and Aedes aegypti: comparison of parent and progeny candidate vaccine viruses. Schoepp R J; Beaty B J; Eckels K H. (College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins.) The American journal of tropical medicine and hygiene, (1990 Jan) Vol. 42, No. 1, pp. 89-96. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB DEN-3 parent (strain CH53489) and progeny candidate vaccine (clone 24/28) viruses were compared in their abilities to interact with Aedes aegypti and Ae. albopictus. The parent and progeny virus were equivalent in their ability to infect, to replicate in, and to be transmitted by both species of mosquitoes. The candidate vaccine DEN-3 clone was **temperature sensitive** and had small plaque morphology. These phenotypic markers remained stable during mosquito passage. Thus, **temperature sensitivity** and small plaque morphology are not reliable biological markers for **attenuation**.

L17 ANSWER 11 OF 20 MEDLINE on STN
87154130. PubMed ID: 3826504. Lack of **attenuation** of a candidate **dengue** 1 vaccine (45AZ5) in human volunteers. McKee K T Jr; Bancroft W H; Eckels K H; Redfield R R; Summers P L; Russell P K. The American journal of tropical medicine and hygiene, (1987 Mar) Vol. 36, No. 2, pp. 435-42. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB A **dengue** type 1, candidate live virus vaccine (45AZ5) was prepared by serial virus passage in fetal rhesus lung cells. Infected cells were treated with a mutagen, 5-azacytidine, to increase the likelihood of producing **attenuated** variants. The vaccine strain was selected by cloning virus that produced only small plaques in vitro and showed reduced replication at high temperatures (**temperature sensitivity**). Although other candidate live **dengue** virus vaccines selected for similar growth characteristics have been **attenuated** for humans, two recipients of the 45AZ5 virus developed unmodified acute **dengue** fever. Viremia was observed within 24 hr of inoculation and lasted 12 to 19 days. Virus isolates from the blood produced large plaques in cell culture and showed diminished **temperature sensitivity**. The 45AZ5 virus is unacceptable as a vaccine candidate. This experience points out the uncertain relationship between in vitro viral growth characteristics and virulence factors for humans.

L17 ANSWER 12 OF 20 MEDLINE on STN
84304706. PubMed ID: 6476215. Selection of **attenuated dengue** 4 viruses by serial passage in primary kidney cells. IV. Characterization of a vaccine candidate in fetal rhesus lung cells. Halstead S B; Eckels K H; Putvatana R; Larsen L K; Marchette N J. The American journal of tropical medicine and hygiene, (1984 Jul) Vol. 33, No. 4, pp. 679-83. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB A strain of primary dog kidney (PDK)-passaged **dengue** (DEN) 4 (H-241) virus cloned by terminal dilution (PDK 35-TD3) was propagated in large volumes in fetal rhesus lung (FRhL) cells to produce a candidate vaccine for evaluation in man. Production seed (FRhL p2) and candidate vaccine (FRhL p3) were subjected to rigorous safety tests to exclude contaminating microbial agents. There was no significant monkey neurovirulence of parental or PDK-passaged DEN-4 virus or of control fluid cultures. FRhL-passaged viruses retained the phenotypic characteristics: small (occasional medium) plaque; **temperature sensitivity** at 38.5 degrees C; and absence of plaque formation in African green monkey kidney cells, cytopathic effect in LLC-MK2 cells, and viral growth in human monocytes. FRhL p2 virus displayed low virulence for monkeys; only one of four animals was viremic and three of four developed low-titered antibody. FRhL p3 virus produced viremia in three monkeys and moderate to high hemagglutination-inhibition and neutralizing antibody titers in all animals. Virus at both passages in FRhL exhibited reduced neurovirulence in suckling mice as compared to parental DEN-4. Because of its safety and desirable monkey virulence attributes PDK 35-TD3 FRhL p3 is recommended for human phase I trial.

L17 ANSWER 13 OF 20 MEDLINE on STN
84304705. PubMed ID: 6476214. Selection of **attenuated dengue** 4 viruses by serial passage in primary kidney cells. III. Reversion to virulence by passage of cloned virus in fetal rhesus lung cells. Halstead S B; Marchette N J; Diwan A R; Palumbo N E; Putvatana R; Larsen L K. The American journal of tropical medicine and hygiene, (1984 Jul) Vol. 33, No. 4, pp. 672-8. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB Two strains of primary dog kidney-passaged **dengue** (DEN) 4 (H-241) virus cloned by terminal dilution (PDK 24-TD3 and 35-TD3) were propagated in fetal rhesus lung (FRhL) cells to produce candidate vaccine virus seeds. Both serial passage and prolonged replication of PDK 24-TD3 in FRhL resulted in appearance of medium and large plaques in LLC-MK2 assays.

monkey-virulent revertants. Serial passage and prolonged replication of PDK 24-TD3 in LLC-MK2 cells did not result in reversion; but, prolonged replication in PDK cells did. Passage of PDK 35-TD3 in FRhL cells resulted in appearance of medium size plaques which, when picked, yielded **temperature sensitive** (ts) (38.5 degrees C) viruses of low monkey-virulence. Because of its stability in monkeys and FRhL cells, reduced monkey virulence and ts property. PDK 35-TD3 is a promising candidate for trial in man.

L17 ANSWER 14 OF 20 MEDLINE on STN
84304704. PubMed ID: 6476213. Selection of **attenuated dengue** 4 viruses by serial passage in primary kidney cells. II. Attributes of virus cloned at different dog kidney passage levels. Halstead S B; Marchette N J; Diwan A R; Palumbo N E; Putvatana R. The American journal of tropical medicine and hygiene, (1984 Jul) Vol. 33, No. 4, pp. 666-71. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB Uncloned **dengue** (DEN) 4 (H-241) which had been passaged 15, 30 and 50 times in primary dog kidney (PDK) cells were subjected to two successive terminal dilution procedures. In the first (3Cl), virus was diluted in 10-fold steps in 10 replicate tubes. An infected tube from a dilution row with three or fewer virus-infected tubes was selected for two further passages. In the second (TD3), virus was triple terminal diluted using 2-fold dilution steps and selecting one positive tube out of 10. Both procedures selected virus population which differed from antecedents. Plaque size of PDK 15 was medium, PDK 30, small and PDK 50, pin-point. PDK 19-3Cl were medium and 56-3Cl, 24-TD3, 35-TD3 and 61-TD3 were all small. All cloned virus replication was completely shut-off at 38.5 degrees C; PDK 15 and 30 continued to replicate at this temperature. Uncloned viruses showed a graduated decrease in monkey virulence with PDK passage; cloned viruses were either avirulent for monkeys (19-3Cl, 56-31Cl, 24-TD3 and 35-TD3) or produced revertant large plaque parental-type viremia (35-3Cl and 61-TD3). Those cloned viruses which exhibited **temperature sensitivity**, reduced monkey virulence and stability after monkey passage may be suitable as vaccine candidates for evaluation in human beings.

L17 ANSWER 15 OF 20 MEDLINE on STN
83159789. PubMed ID: 6832818. **Temperature-sensitive** events during the replication of the **attenuated** S-1 clone of **dengue** type 2 virus. Eckels K H; Summers P L; Russell P K. Infection and immunity, (1983 Feb) Vol. 39, No. 2, pp. 750-4. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB **Temperature-sensitive** events occurring during the replication of the **attenuated** S-1 clone of **dengue** type 2 virus were examined. The S-1 clone was more thermolabile than the parent virus at the nonpermissive temperature of 38.5 degrees C. Adsorption experiments in fetal rhesus monkey lung cells revealed an inefficient adsorption of S-1 at 38.5 degrees C compared with the parent virus, suggesting an alteration in a thermolabile virion protein important in adsorption. The production of S-1 viral RNA and antigen occurred at the nonpermissive temperature, which indicated that early events in the replication cycle of S-1 were not affected. Release of infectious virus at 38.5 degrees C was not impaired; however, lower amounts of infectious virus in infected cells at the nonpermissive temperature indicated that maturation of the S-1 clone was suppressed.

L17 ANSWER 16 OF 20 MEDLINE on STN
83072305. PubMed ID: 7149108. **Dengue**-2 vaccine: oral infection, transmission, and lack of evidence for reversion in the mosquito, Aedes aegypti. Miller B R; Beaty B J; Aitken T H; Eckels K H; Russell P K. The American journal of tropical medicine and hygiene, (1982 Nov) Vol. 31, No. 6, pp. 1232-7. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB The **dengue**-2 vaccine virus (S-1), and its parent virus (PR-159), were compared for their ability to infect orally, to replicate in, and subsequently to be transmitted by Aedes aegypti mosquitoes. The vaccine virus was markedly less efficient in its ability to infect mosquitoes orally. After ingesting infectious bloodmeals containing 3, 7 to 8.2 log10MID50/ml of the respective viruses, 56% (220/396) of the mosquitoes became orally infected with the parent virus contrasted with 16% (66/397) for the vaccine virus. None of the 16 infected mosquitoes transmitted the vaccine virus, while 14% (3/22) of the mosquitoes transmitted the parent virus. The vaccine virus remained **temperature-sensitive** (restrictive temperature 39 degrees C) after orally infecting and replicating in Ae. aegypti mosquitoes.

L17 ANSWER 17 OF 20 MEDLINE on STN
83072304. PubMed ID: 7149107. **Dengue**-2 vaccine: infection of Aedes aegypti mosquitoes by feeding on viremic recipients. Bancroft W H; Scott R M; Brandt W E; McCown J M; Eckels K H; Hayes D E; Gould D J; Russell P K. The American journal of tropical medicine and hygiene, (1982 Nov) Vol. 31, No. 6, pp. 1229-31. Journal code: 0370507. ISSN: 0002-9637. Pub. country:

AB Colonized Aedes aegypti mosquitoes were fed on voluntary recipients of an experimental, live, **attenuated, dengue** type 2 (PR 159/S-1) vaccine to estimate the frequency of vector infection and the stability of the virus in mosquitoes. Two volunteers were viremic at the time of mosquito feeding, but only two of 114 mosquitoes that took a viremic blood meal became infected with the vaccine virus. Strains of virus recovered from the bodies of the mosquitoes and the volunteer's blood retained the **temperature sensitivity** and small plaque growth characteristics of the vaccine virus. **Dengue** viral antigen was not detectable in any of the mosquito heads by direct immunofluorescence and in vitro virus transmission by droplet feeding was not observed. This experiment showed that vector mosquitoes can be infected with vaccine virus by feeding on viremic vaccinees. Furthermore, the virus is sufficiently stable to retain the in vitro growth characteristics associated with the vaccine virus.

L17 ANSWER 18 OF 20 MEDLINE on STN
81166976. PubMed ID: 7216469. **Dengue**-2 vaccine: virological, immunological, and clinical responses of six yellow fever-immune recipients. Bancroft W H; Top F H Jr; Eckels K H; Anderson J H Jr; McCown J M; Russell P K. Infection and immunity, (1981 Feb) Vol. 31, No. 2, pp. 698-703. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB Six male volunteers, previously immunized with yellow fever vaccine, were inoculated subcutaneously with a live, **attenuated dengue**-2 virus (PR-159/S-1) candidate vaccine. Five recipients developed viremia 8 or 9 days after vaccination, which lasted 1 to 10 days. The onset of viremia was followed by fever in three people, transient leukopenia in four, and an erythematous rash in one. One volunteer developed an oral temperature of 38.8 degrees C with headache, myalgia, fatigue, and photophobia suggestive of mild **dengue** fever. All five viremic volunteers developed fourfold or greater rises in serum neutralizing antibody. The sixth volunteer, who had a low titer of preexisting **dengue**-2 neutralizing antibody, had no viremia, no symptoms, and a modest rise in hemagglutination inhibiting antibody. Virus isolates obtained from plasma retained the small-plaque and **temperature-sensitive** growth characteristics of the vaccine virus in vitro. In this study, the vaccine virus genetically stable and immunogenic and seemed sufficiently **attenuated** for additional testing in humans.

L17 ANSWER 19 OF 20 MEDLINE on STN
78004976. PubMed ID: 409682. Virulence and immunogenicity of a **temperature-sensitive dengue**-2 virus in lower primates. Harrison V R; Eckels K H; Sagartz J W; Russell P K. Infection and immunity, (1977 Oct) Vol. 18, No. 1, pp. 151-6. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB Clones of **dengue**-2 virus were tested for virulence by inoculation of rhesus monkeys and chimpanzees. Although primates showed no overt signs of illness, inoculation with the parent virus or a subline of a large-plaque clone resulted in a viremia lasting 1 to 7 days. By these criteria, sublines of a small-plaque clone were significantly less virulent and produced little or no viremia in primate hosts. Although they had a substantially reduced viremia, primates inoculated with the small-plaque sublines showed stimulation of complement-fixing, hemagglutination-inhibiting, and neutralizing antibodies. The protection afforded rhesus monkeys 3 months after inoculation with two of the small-plaque sublines was demonstrated by a lack of viremia and a failure to escalate preexisting antibody levels after challenge with the parent virus. Both the S-1 subline and the parent virus had a limited capacity to produce central nervous system pathology in monkeys inoculated intrathalamically and intrathecally. Evidence thus far accumulated for primates indicates that the S-1 subline of **dengue**-2 virus has potential value as a candidate vaccine virus.

L17 ANSWER 20 OF 20 MEDLINE on STN
76189294. PubMed ID: 57925. Chemically induced **temperature-sensitive** mutants of **dengue** virus type 2: comparison of **temperature sensitivity** in vitro with infectivity suckling mice, hamsters, and rhesus monkeys. Tarr G C; Lubiniecki A S. Infection and immunity, (1976 Mar) Vol. 13, No. 3, pp. 688-95. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB A series of **temperature-sensitive** (ts) mutants of **dengue** virus type 2 (DEN-2, TH-36 isolate) were induced by treatment with 5-azacytidine. These mutants and parental viruses were compared for the ts trait and/or **attenuation** in four systems: primary hamster kidney cells, suckling mice, golden Syrian hamsters, and rhesus monkeys. Seven clones judged to possess the ts trait in virto demonstrated a variety of patterns in vivo. On initial isolation, five of seven ts mutants exhibited reduced mouse lethality. The remaining two mutants possessed parental levels of mouse lethality. In hamsters, neither ts mutant nor parental viruses replicated very well, and then only when inoculated intracerebrally. Studies in rhesus monkeys indicated that all seven ts clones and parental viruses

to produce detectable viremia. After challenge with parental virus, all vaccinated monkeys demonstrated rapid secondary-type antibody response. Reversion from ts to ts(+) was confirmed to ts-1 in mice and ts-3 in monkeys, and was strongly suspected in several other instances.

```
=> d his

     (FILE 'HOME' ENTERED AT 01:43:33 ON 10 JUL 2006)

     FILE 'USPATFULL' ENTERED AT 01:43:53 ON 10 JUL 2006
                E WHITEHEAD STEPHEN S/IN
L1           14 S E3
                E MURPHY BRIAN R/IN
L2           32 S E3
L3            3 S L2 AND DENGUE
                E HANLEY KATHRYN A/IN
L4            1 S E3
                E BLANEY JOSEPH E/IN
L5            2 S E3-E4
L6            0 S L5 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 01:48:19 ON 10 JUL 2006
                E WHITEHEAD S S/IN
L7            9 S E3
L8            9 S L7 NOT (L1 OR L2)

     FILE 'MEDLINE' ENTERED AT 01:50:04 ON 10 JUL 2006
                E WHITEHEAD S S/AU
                E WHITEHEAD STEPHEN/AU
L9           22 S E4-E5

     FILE 'USPATFULL' ENTERED AT 01:53:31 ON 10 JUL 2006
L10        2809 S DENGUE
L11        1267 S L10 AND ATTENUAT?
L12          95 S L11 AND (TEMPERATURE-SENSITIV?)
L13          17 S L12 AND NS5

     FILE 'MEDLINE' ENTERED AT 01:56:04 ON 10 JUL 2006

     FILE 'MEDLINE' ENTERED AT 01:56:41 ON 10 JUL 2006
L14        5188 S DENGUE
L15         196 S L14 AND ATTENUAT?
L16          22 S L15 AND (TEMPERATURE-SENSITIV?)
L17          20 S L16 NOT L13

=> log y
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                 6.35     102.04

STN INTERNATIONAL LOGOFF AT 01:59:55 ON 10 JUL 2006
```